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V.

I. EXPERIMENTS ON THE EFFECT OF FREEZING AND OTHER LOW TEMPERATURES UPON THE VIABILITY OF THE BACILLUS OF TYPHOID FEVER, WITH CONSIDERATIONS REGARDING ICE AS A VEHICLE OF INFECTIOUS DISEASE.

II. STATISTICAL STUDIES ON THE SEASONAL PREVALENCE OF TYPHOID FEVER IN VARIOUS COUNTRIES AND ITS RELATION TO SEASONAL TEMPERATURE.

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TABLE OF CONTENTS.

PART I.

PART II.

PART III.

PART I.

I. INTRODUCTORY.

In view of the fact that the micro-organism which is commonly considered to be the cause of typhoid fever appears to be able to survive for longer or shorter periods in the environment of man, it becomes important to discover, as nearly as may be, its behavior under various natural conditions. Some knowledge of this kind we have already in the case of heat and light; some, also, in respect to low temperatures under certain conditions. But a careful review of the present state of our knowledge in regard to the influence of cold upon the bacillus of typhoid fever shows that much still remains to be done in order to make our knowledge in this direction more precise.

The subject assumes great practical importance when we begin to consider the influence of external conditions upon the longevity of the bacillus in nature, particularly in those regions in which there is a considerable variation of climate. It was a theory formerly widely held that the specific organism of typhoid fever was not only capable of enduring for a long time outside the human body, but even that a residence in earth, filth heaps, and the like was an essential phase in its life history. Modern researches have thrown grave doubt upon this earlier theory, but at the same time rigid inquiry into epidemics and further knowledge of the disease itself have shown how readily the micro-organism may become widely distributed in the environment. Prolonged and careful studies of the influence of temperature upon the bacillus of typhoid fever, have led us to believe that this factor plays a part in the seasonal distribution of the disease which is of the highest importance, making it possible to explain, by the co-operation of this and other factors, such as light and dryness, certain phenomena hitherto inexplicable or little understood. An obvious and direct application of the principles worked out concerns one of the principal food supplies of man, and an important section of the following paper is therefore devoted to a consideration of the danger of the conveyance of the disease in question by polluted ice.

II. A REVIEW OF THE LITERATURE RELATING TO ICE AS A VEHICLE OF DISEASE AND TO THE BACTERIOLOGY OF ICE.

A. INFECTIOUS DISEASES ATTRIBUTED TO POLLUTED ICE AND ICE-CREAM.

THE interest of the authors in this subject was first aroused by the practical questions connected with ice supply and the public health. As will appear in the paragraphs immediately following, diseases, and particularly typhoid fever, have not infrequently been attributed to impure ice.

The first outbreak of disease directly ascribed to this source was reported in this country in 1875,[1] at the summer resort of Rye Beach. Dr. Nichols of Boston, who was called in to investigate the affair, found the illness, a more or less severe intestinal disorder, confined to the guests of one of the two large hotels of the place. The other hotel and adjacent cottages were unaffected. The milk and water supplies and the drainage appeared above suspicion. The ice for the hotel, however, was cut on a small pond whose waters were rendered very foul by a mass of putrescent matter, composed of marsh mud and decomposing sawdust. A chemical analysis of the ice, and of the water from the pond, showed high total organic matter and high ammonia, both free and albuminoid. Three cases of the disease outside the hotel directly following the use of this ice made the evidence still stronger. Three years later Dr. Smart, U. S. A.,[2] attributed some cases of a "malarial remittent fever" in a Rocky Mountain army post to the contamination of mountain streams by melting snow. The high organic content of the water in early spring was probably due to this cause, and he believed that the "*materies morbi*" of malaria had a similar origin. In the summer of 1879 an outbreak of dysentery occurred in Connecticut which is discussed in the Second Report of the Board of Health of that State.[3] Out of the eleven persons, including the family residing in a certain farmhouse, two hired men, and relatives who came to assist in nursing, there were eight cases of dysentery, three of them fatal, and two cases of persistent diarrhœa. The drinking water in use gave satisfactory results on analysis, but the soil adjoining the house was damp and polluted, and the ice used came from a small stream which served as a running place for pigs. Analysis of the ice-water showed high ammonias, and this appeared to the

investigators the most probable cause of the disease. In the Report of the same Board for 1882,[4] an interesting single case of typhoid fever is cited as probably derived from ice. The patient had lived alone for some months in a house whose sanitary conditions were apparently perfect. He was inordinately fond of ice-water, and the ice for his house was cut on a small pond near by. It appeared on investigation that the drains from some laborers' houses emptied directly into the pond, and that in these houses there had been three cases of typhoid fever during the previous summer. Attention was also called to the general danger from ice supply, by the Connecticut State Board of Health in 1880, by the Massachusetts State Board in 1876 and 1889, by the Michigan Board in 1882 and 1884, by the New Hampshire Board in 1882, the New York Board in 1886, the Minnesota Board in 1886, and the sanitary authorities of Chicago in 1896 and of Milwaukee in 1876.

Duclaux[5] appears to have been the first European to give the matter marked attention, although a recent French writer[7] mentions an ice epidemic at "Eveshem," in 1882, of which we have found no other account. Duclaux enlarged at length upon the danger from ice, especially the artificial ice made in Paris from the water of certain highly polluted canals. In 1893 Professor Riche[6] made a long report to the *Conseil d'hygiène et de salubrité de la Seine* upon the dangers to the inhabitants of Paris from the sale of highly polluted ice. He quoted a letter from Pasteur as follows: "Le docteur Roux vous a dit son opinion, et c'est aussi la mienne, que toute eau impropre à la boisson l'est également pour préparer, en hiver, de la glace pour l'alimentation. Les microbes inoffensifs ou pathogènes résistent presque tous à des températures même très basses." M. Riche showed that much of the Paris ice came from contaminated sources, and recommended strong legal restrictions upon its sale. Finally, Dr. Dorange, in the *Revue d'Hygiène*,[7] described a supposed ice-epidemic of typhoid fever at the military post of Rennes in the autumn of 1895. Eight lieutenants of the regiment there stationed were taken ill between the twelfth and the twenty-fifth of December. The fact that these officers did not habitually live in common but had all been present at a regimental banquet upon the fourth of December, pointed to that occasion as the moment of infection. The higher officers dined in a separate room, and used no water but the town supply, which was excellent. The lieutenants, on the other hand, drank a "tisane" of champagne mixed with chilled water. The man who provided this claimed that it also was derived from the regular town-supply. The fact that the town water could be obtained by him only from a considerable distance and under strict police regulations, led Dr. Dorange to suspect that he had made use of the water in a reservoir which stood in the room

where he cooled his decanters and which received the meltings from his stock of ice. The ice supply of the town was considered highly polluted. The additional facts are cited that the menus of the different classes of officers were the same, and that certain of the petty officers who did not drink from the "tisane" but made use of beer instead, escaped the disease.

Altogether it appears probable that the milder intestinal disorders, caused by mere decomposing organic matter and not by specific germs, have at times been caused by polluted ice. The Rye Beach epidemic was carefully and thoroughly studied, and leads directly to that conclusion. With respect to typhoid fever the case is different. The only ice-epidemic of typhoid fever which has come to our notice, viz., that at Rennes, rests on a doubtful chain of circumstances, and lacks the confirmation of a complete exclusion of all possible factors other than ice. We have been unable, then, to find any conclusive evidence that typhoid fever has been caused by polluted ice-supply.

A number of English epidemics of typhoid fever, more or less clearly traced to ice-cream, should be noticed here, although the conditions are quite different from those which obtain in the case of ice. The first of these epidemics occurred in the English sanitary districts of Greenwich and Rotherhithe in 1892.[8] During the last week of September and the two months next following 511 cases were reported, the beginning of the attack in 15 per cent of the cases falling on October 1 and in 57 per cent of the cases falling in the fortnight preceding October 3. A remarkably large proportion of the victims were young children. The water supply and sewerage of the four separate foci of infection were different and apparently all in good condition. The milk supply of the households attacked came from seven dairy farms, and in many cases consisted only of condensed milk. Suspicion was then directed to the ice-cream sold by Italians from barrows in the street. A careful canvass of one neighborhood in which 56 cases of typhoid fever had occurred showed that 924 persons lived in houses where ices had not been eaten, 232 lived in houses where ices had been obtained from shops, and 395 in houses where ices had been obtained from a certain ice-cream vendor. All the cases of typhoid fever were in this latter class. A detailed examination of the cases in all the infected areas showed that 88.9 per cent of the sufferers had eaten ices, and that, of these, 91.4 per cent had obtained their supply from ten Italian vendors living in a certain Mill Lane, of whom one was the dealer above mentioned. The sanitary conditions in Mill Lane were found to be abominable; and in the family of one of the purveyors of ice-cream two children had sickened with typhoid fever on July 29 and August 5 respectively.

An epidemic of typhoid fever which attacked over 800 persons in the county of Renfrew, in Scotland, in 1893, was attributed by Dr. A. C. Munro partly to ice-cream and partly to the public water-supply.[9] Out of the first 180 cases 63 were shown to have eaten ice-cream prepared by a dealer in whose family a case of typhoid fever had occurred during the previous month. The patient had been in intimate contact with the ice-cream business during the greater part of her illness.

Vaughan and Perkins, in 1895,[10] ascribed two epidemics of severe, but not fatal, intestinal disease to a new pathogenic bacillus which they isolated from ice-cream in one case and from cheese in the other. The germ belonged to the colon group, and the authors note that neither twenty-nine days of continuous freezing nor alternate freezing and thawing could destroy its vitality.

Dr. Hope, in 1898,[11] studied an epidemic affecting 27 school children in Liverpool in which the only clue appeared to be the presence of all the patients at a fair just at the time of infection. Here 24 of the children had eaten ice-cream and two more had partaken of "chip" potatoes sold by an Italian in whose house there had been two cases of typhoid fever.

In these cases of infection from ice-cream there is, of course, no certainty that the disease germs were actually frozen. The possibility of contamination from spoons, vessels, and the hands of the vendor might easily account for all the phenomena. Even if the infection was really carried in the ice-cream the exposure to a low temperature must have been a relatively short one. The same reasoning applies to the famous Plymouth, Pa., epidemic of typhoid fever. This little mining town had 1200 cases of the disease and 130 deaths among its 8000 inhabitants in 1885, and the investigation[12] clearly traced the infection to the dejecta of a single typhoid fever patient which were thrown out on the snow on the banks of the brook supplying the town with water, and which had been washed in by the first general thaw of the spring. It may easily have been that the discharges thrown out during the day or two preceding the thaw were never really frozen at all. In any case the conditions affecting germs imbedded in a solid mass of rich food material are quite different from those which obtain in the formation of ice upon a stream or pond.

B. BACTERIA IN NATURAL ICE, SNOW, AND HAIL, AND IN ICE-CREAM.

In spite of the absence of epidemiological evidence, it has been the common opinion of sanitarians that ice might be an important source of infection for typhoid fever or any other germ disease. Its apparent purity was shown by the earliest bacteriologists to be deceptive. Burdon-Sanderson,[13] in 1871, found that liquid

culture media showed bacterial growth when inoculated with melted ice or with snow. In the next year, Cohn[14] described experiments in which nutrient solutions containing bacteria were not sterilized by exposure to a temperature ranging as low as −18° C. for about 6 hours or by a temperature with a minimum of −7° C. for 18 hours.

Professor Joseph Leidy, in 1884,[15] exhibited, at a meeting of the Academy of Natural Sciences at Philadelphia, snow water derived from melted ice, containing not only Infusoria but also Rotifers and Worms. Pohl, in the same year,[16] recorded the finding of many bacteria in snow and ice, 110 per centimeter in Neva ice, and 20,774 in one sample of bubbly ice. He also found bacteria in falling snow, the number decreasing with the continuation of the storm. A report on the ice supply of the city of Syracuse[17] was made to the New York Board of Health in 1886 in which the presence of a great number of bacteria was noted in ice from Onondaga Lake and the Erie Canal. In 1888 Breunig[18] found 1310–2760 germs in ice, and Kowalski[19] analyzed sixty samples of natural ice, and found from 10 to 1000 germs per cubic centimeter, no sample being sterile. Still another paper was published at this period, 1888–89, by Heyroth,[20] who studied the Berlin ice-supply, and, in 25 samples, found from 2 to 133,000 bacteria per cubic centimeter, the highest figures corresponding to chemical analyses which showed the most marked pollution. An elaborate report was made by the State Board of Health of Massachusetts in 1889,[21] in which 238 samples of natural ice from the ponds and streams of this State were analyzed bacteriologically. The figures for ice from different portions of the cake were as follows: —

	Number of Samples.	Bacteria per c.c.		
		Maximum.	Minimum.	Average.
Transparent Ice	27	893	0	105
Clear Ice	75	370	0	15
Bubbly Ice	113	1950	0	111
Snow Ice	23	2968	0	622

A "Lancet" analytical sanitary commission made an examination of some ice sold in London in 1893, and found that while all the specimens gave good chemical analyses, two out of the six examined contained 400 to 700 bacteria per cubic centimeter.[22]

Girard and Bordas[23] published some startling analyses of the Paris ice-supply also in 1893. They found a minimum of 23,000 colonies and a maximum of 100,000 colonies per cubic centimeter, including the Bacillus coli communis and a patho-

genic vibrio. These quantitative results are so large as to suggest that the samples were probably not planted promptly after melting.

Christomonas[24] has recently studied artificial ice, and reports that when water containing 71 bacteria per centimeter was frozen, 450 germs per centimeter were found in the central core and 8–10 in the clear ice at the sides.

The bacteria of snow and hail have also received considerable attention. Soon after the work of Pohl,[16] Janowsky[25] made analyses of old and of freshly fallen snow in the neighborhood of Kiew, and found bacteria in both, less in the former than in the latter. Schmelk[26] studied the bacterial life in the snow of a Norwegian glacier and in the chill streams flowing therefrom; and in a later paper[27] he recorded small numbers in both snow and ice at Christiania. Bujwid[28] found 21,000 bacteria per cubic centimeter in the analysis of a melted hail-stone; and Foutin[29] in Russia obtained similar, though smaller, figures.

Giacosa[30] found bacteria present in small numbers in snow lying at an elevation of 3800 meters above the sea, and Abbott[31] noted 703 colonies per cubic centimeter in hail. Dominguez,[32] in 1892, published a paper on the bacterial content of hail; and finally, Scofone,[33] who accompanied a scientific expedition to Monte Rosa in 1894–95, recorded the presence of small numbers of bacteria in melted snow obtained from high altitudes. In the following year he gave the results of some examinations made on a plateau 2460 meters above the sea, which confirmed his previous conclusion that the bacteria in the deeper layers of the snow were somewhat more numerous than in the superficial layers.[34]

The number of bacteria present in ice-cream has been shown at times to be enormous. Klein[35] found the germ content of London ice-cream very high, and B. coli communis frequently present. Nield-Cook[36] recorded from 5,000,000 to 14,000,000 germs per cubic centimeter in ice-cream from the same source, the majority being colon bacilli. Stevenson[37] testified, at the trial of an Italian ice-cream vendor, that he had found over 4000 germs per cubic centimeter, of which three proved to be B. coli communis. Wilkinson[38] reached similar results, and quoted, without reference, the following results of other observers: —

Macfadyen	119,000 — 7,000,000	bacteria per cubic centimeter.
Kanthack	8,000,000 — 13,000,000	" " " "
Foulerton	500,000 — 7,000,000	" " " "

In this connection it may be interesting to note the very small numbers of bacteria present in the air and water of the Arctic regions. Nystrom[39] discovered this fact in 1868 by the exposure of a number of flasks of putrescible matter, after the

manner of Pasteur. Couteaud[40] found but one colony in 19 flasks exposed to Arctic air, the experiment being carried on, however, on the open sea, so that the result is not surprising. He also found but few species present in some analyses of water and of soil. In the Nansen expedition the poverty of the bacterial flora of the air was noted. Finally, Dr. Levin[41] of Stockholm made an elaborate study of the subject with the Natthorst expedition. In 21,600 liters of air examined at twenty different places 3 germs alone were found, all in one sample. In sea water, at the surface, 11 germs per centimeter occurred, belonging apparently to two characteristic species. Fresh water and melted ice and snow gave similar small numbers. Samples from considerable depths in the ocean showed somewhat higher numbers than were obtained at the surface. Finally, tests of the alimentary canals of various Arctic animals and birds showed many of them to be completely sterile.

C. EXPERIMENTS ON THE EFFECT OF FREEZING AND OTHER LOW TEMPERATURES UPON THE VIABILITY OF BACTERIA.

Laboratory experiments have confirmed the conclusion, drawn from the examination of natural ice, that freezing is by no means always fatal to germ life. Von Frisch[42] froze putrefying solutions and reduced the frozen mass to a temperature of −87° C., and after some hours found that sterilization had not ensued.

Pictet and Young[43] subjected bouillon cultures of several species to a temperature below −70° C. for 108 hours, during twenty hours of which time the temperature was below −130°. After this treatment B. anthracis and the bacillus of "charbon symptomatique" were alive and virulent; B. subtilis and B. ulna grew readily; half the inoculations made from the cultures of two species of micrococci grew and half did not. Finkler and Prior[44] stated that the vibrio described by them could survive a temperature of −4° C. for many days. McKendrick,[45] in a communication to the British Association in 1885, noted that putrescible liquids were not sterilized by a temperature of −84° C. Forster[46] found that the phosphorescent bacteria which he isolated from fish preserved by cold storage grew vigorously at 0° C. Fischer[47] isolated 5 species of bacteria from the water of the harbor at Kiel, and 9 other forms from the soil, all capable of multiplying at 0°. In the research already cited,[20] Heyroth froze gelatine stick-cultures of various species for from seven to ten days, and then placed them once more under favorable conditions; out of 30 species, thus treated, 25 showed growth, though 5 of these had partially lost their liquefying power. D'Arsonval,[48] in 1891, recommended liquefied carbonic acid for use in sterilizing organic extracts, and stated that when the treatment is prolonged, especially

if broken by a return to 40° for a time, "nothing living can resist it," but his own and other later researches showed the error of this conclusion. Forster, in 1892,[49] examined various natural waters, foods, wastes, sweepings, and soils for bacteria capable of growth at 0°, and found a few such forms in water, earth, and street sweepings. When present at all they occurred in great numbers. Forster also demonstrated the multiplication of bacteria and the progress of decomposition in butcher's meat chopped up and kept in an ice calorimeter. Fischer[50] noted that Miller's vibrio and the vibrio of Finkler and Prior could withstand a freezing temperature for some days.

Pictet, in 1893,[51] studied the effect of cold on plants and animals of the most widely separated classes. Of the bacteria he subjected 30 to 35 species to temperatures ranging as low as −200° C. by immersing them in liquid air, but the viability of the germs used appeared unaffected after "prolonged" treatment of this sort. D'Arsonval and Charrin[52] subjected cultures of Bacillus pyocyaneus to a temperature of −40° to −60° C. with the result that, in six out of eight instances, the germs remained alive.

In another paper[53] these authors mentioned that Bacillus pyocyaneus after exposure to −40°, −60°, and −95° C. exhibited profound changes in morphology and physiology. For some generations the descendants of the frozen germs showed elongated, ovoid, and other abnormal forms, and their colonies on gelatine were also of unusual character. Weber[54] noted that Hofer's bacillus, producing a contagious disease among Crustacea, can endure a temperature of −40° C. for four hours, as well as repeated thawings and freezings.

Professor Mason[55] recorded the exposure of cultures of "ordinary bacteria" to the temperature of solid carbon dioxide for many hours without causing their destruction. Still more recently Ravenel[56] submitted cultures of the anthrax, diphtheria, and typhoid bacilli, and of Bacillus prodigiosus to the temperature of liquid air, 191° below zero Centigrade, for periods of three hours, thirty minutes, one hour, and one hour respectively; in no case could any weakening of the vegetative power of the culture be detected.

Besides Pictet and Young[43] and Ravenel[56] a number of other observers have tested the effect of low temperatures upon specific pathogenes. Cadéac and Malet[57] found that tuberculous matter kept frozen for four months still produced characteristic symptoms in guinea pigs. In some work on the spores and vegetative forms of Bacillus anthracis carried out by one of the Franklands and Dr. Templeman,[58] it was found that a single freezing at −20° C. reduced the numbers present in water from

15,000 to 3500 per cubic centimeter, and after 29 successive freezings, extending over a period of three months, 3000 germs per centimeter could still develop. Evidently the vegetative forms were killed by one freezing, and the spores, not at all. Another culture which was spore-free showed reduction from 8000 germs per centimeter to 2 per centimeter after one freezing, sterilization following the second freezing.

Gabritschewsky, Wladimiroff, and Kressling and Gladin quoted by Kasansky[59] found that the plague germ could bear an artificial cold of −22° C. for two hours and natural cold ranging from 0° to −20° C. for from twelve to forty days. Kasansky himself in 1897–98 made some interesting experiments on the resistance of the specific organisms of plague and diphtheria against cold. The cultures were placed outside the window of the laboratory at Kasan, sheltered from light but exposed to the winter's cold, which ranged from a maximum of 5° C. to −34° C. Bouillon cultures of the plague germ showed life after thirty-two days; four months' exposure sterilized most of the tubes, but in one case growth was obtained after six months. Of the agar cultures tested some died in four months, and others contained living germs after five months and a half. Sixteen bouillon tubes of the diphtheria bacillus were kept for six months under similar conditions, and one tube only showed growth at the end of that time; two of the others, however, still gave positive results on the fifty-third and one hundred and eighteenth day, respectively.

Abel[60] exposed cultures of the diphtheria germ on blood serum and on dried threads to the winter's cold at Greifswald, and compared them with cultures kept in the room in the same condition. The first race used persisted on the blood serum for the whole period of eighty-six days both in the room and out of doors, although in the second case the growth obtained was meagre after the fiftieth day. The dried germs had disappeared by the sixty-eighth day out of doors and by the seventy-fourth indoors. Of the second race the serum culture remained alive in the room all through the experiment; the frozen one showed no growth after the seventy-fourth day. The threads gave living germs up to the seventy-fourth day in-doors and up to the fifty-sixth day out-doors. The threads of the third race gave precisely the same result; the serum cultures kept in the room gave vigorous growths up to the end of the experiment, while only two colonies developed from the inoculation of the frozen tube. The out-door temperature during the experiment varied from 12° C. to − 20° C.

With regard to the behavior of the typhoid bacillus in ice, there is more evidence available. Dr. Carl Seitz[61] noted in 1886 that cultures of this organism in gelatine, bouillon, and milk were not rendered sterile by the continuance of a temperature

below 3° C., although the growth on gelatine at the low temperature was very much retarded. Dr. Billings, in this country,[62] described a single experiment in which five cubic centimeters of sterile water were inoculated with the typhoid germ and frozen by the out-door cold. On the next day the frozen mass was thawed, and three gelatine tubes and one agar tube were inoculated with portions of it. Three of the four tubes showed typical growths. Chantemesse and Widal[63] recorded the freezing of bouillon cultures of the same microbe without sterilization. Bashenow[64] stated that typhoid germs survived exposure for thirteen days to a temperature between −8° and −15° C. Janowsky published in 1890 some very extended researches[65] in which he used pure cultures of the typhoid bacillus in bouillon and froze them by means of ice and salt, ice and chloride of calcium or carbon dioxide and ether. He made no quantitative estimations; but bouillon frozen by each of the above methods could still produce growth in Esmarch roll-tubes. Janowsky tried also the effect of successive freezings, using the calcium-chloride mixture. After the culture had solidified, it was left in the freezing mixture for fifteen minutes, then thawed in a water bath at 25°–30° C., a sample taken, and the cycle repeated. This was done three times a day; and during the night the culture was kept at 2°–5° C. After twelve such freezings sterilization had not been accomplished; the development of the frozen bacilli was, however, much retarded. To imitate more closely the conditions in nature, Janowsky placed a bouillon culture and two flasks in which were threads bearing the germ in a dried condition, in a wire cage out of doors. Four sets of experiments were conducted, in three of which periods of seven, ten, and twelve days, respectively, did not suffice for sterilization. In the fourth set of cultures the bouillon tube showed no growth after nineteen days; the minimum temperature during the period had been −17° C. and the maximum 4°, the culture thawing and freezing three times. Finally, among experiments on the typhoid bacillus must be mentioned a remarkable paper by Remlinger,[66] in which he states that he used a culture of B. typhi of such virulence that .5 c.c. would kill a guinea pig in 36–48 hours. He took agar cultures of this germ out of the incubator every two or three hours to immerse them in water, cooled down to 22°–23°, for ten minutes. After ten days of this treatment the cultures had entirely lost their virulence, and after thirty-five days their power of growth as well. The author does not state whether control experiments were made or not.

Even more extensive is the literature with respect to the effect of cold on the cholera vibrio. Koch, the discoverer of the organism, stated that it was not destroyed by a temperature of −10° C. in ten hours.[67] Raptschewski[68] found that cholera germs could endure for a month severe cold, ranging as low as −15° C., but that a tempera-

ture of −21° C. was fatal. Von Babes[69] succeeded in keeping a series of agar cultures of the vibrio alive, though exposed to the cold of a Berlin winter (1884–85) ranging as low as −14° C. In the year 1893 no less than eight papers were published dealing with the relation of the cholera germ to cold. Schruff[70] found that a broth culture made from fresh choleraic fæces was not sterilized by eight months' exposure to the winter's cold ranging as low as −12.5° C. Finkelnburg[71] noted that cultures of an old laboratory race were killed out in ten days, while cultures of fresher races were not.

Karschinski[72] stated that a cholera culture with which he worked was sterilized in four days by an average cold of −12.7° C. with a minimum of −17.6° C. Renk[73] froze the germs in sterilized river water at −5° C. to −7° C. and kept the flasks at that temperature, removing one each day for examination. Growth resulting from the melted ice was tested by cover-glass examination and by the Indol reaction. After five days' uninterrupted freezing the cholera germs disappeared, but when the period was broken by the melting of the contents of a flask for analysis and its re-freezing, a little longer period was necessary. When unsterilized river water was inoculated and frozen, the bacteria present fell off from 1,483,000 per centimeter to 62,445 in twenty-four hours, and to 4480 after three days. The cholera germs in this case could not be detected after seventy-two hours, and in one case not after thirty-nine hours. Uffelmann[74] found that cholera germs died out in five days at −15.5° C. and in three days at −24.8° C. Wnukow,[75] on the other hand, stated that gelatine stick cultures of the same microorganism were subjected for forty days to an outdoor temperature between −1° C. and −32° C. without sterilization. Double thawing and freezing also failed to destroy their power of growth. Montefusco[76] tested the pathogenicity of chilled cholera cultures for guinea pigs, and recorded that a temperature of −10° to −15° C. entirely destroyed their virulence in half an hour, while a temperature between 0° and −5° only weakened it. Cultivation at 37.5° soon restored the powers of the germs, but in the chilled and attenuated condition they produced a state of immunity in the animals injected. Abel[77] also mentions experiments in which cholera vibrios frozen in bouillon died out completely in from three to eight days. Kasansky,[78] in 1894, found that cholera cultures withstood for four months the winter's cold at Kasan, where the temperature fell to −31.8° C. One culture gave growth after twenty days of freezing. Some were thawed and refrozen as many as twelve times. After longer exposure, for five months, the cultures gave no growth. Kasansky demonstrated nearly as great a resistance to cold in the case of the vibrios of Finkler-Prior, Miller, Deneke, and Metschnikoff. Finally, some light was thrown on the discordant results of previous observers by the work of Weiss,[79] who inoculated tubes of broth and water from

the Spree with cholera cultures and froze them, thawing, sampling, and refreezing the tubes daily. In broth the germs persisted for twenty-one days, but in river water only for five days, the addition of a little broth to the water prolonging the time to eight days. Fresh intestinal contents of a cholera patient showed no vibrios after two or three freezings.[1]

From this long series of experiments it is evident that sterilization of rich cultures of bacteria cannot always be secured by the action of even very extreme cold. Hence the conclusion was drawn that the freezing of water could not be trusted at all to remove its bacterial impurities. There are, however, two objections to this line of reasoning. In the first place, the effect of cold on germs suspended in water may differ materially from its action on similar organisms when in a richly nutrient medium. In the second place, even if sterilization does not result from freezing in cultures containing millions of bacteria, it is conceivable that such a large proportion of the microbes may perish as to render very slender the chance of danger from ice formed under natural conditions. Experiments have shown that easily detected germs like B. prodigiosus can pass through a sand filter when applied to the surface in large numbers under certain conditions; yet a sand filter, in practice, is regarded as an efficient protection. A quantitative determination of the percentage reduction actually effected by freezing is required before drawing conclusions as to the sanitary significance of ice-supply in relation to the public health.

D. QUANTITATIVE STUDIES UPON THE DESTRUCTION OF BACTERIA BY FREEZING AND OTHER LOW TEMPERATURES.

The quantitative studies of Frankland [58] on B. anthracis, of Renk [73] on river-water bacteria, and of Christomonas,[24] on artificial ice, have already been mentioned. Work on the disappearance of bacteria in the freezing of natural water had, however, been undertaken at a much earlier period. Pengra,[80] in 1884, made an actual microscopic count of the organisms present, working with bacteria (species not stated), and other micro-organisms from decomposing meat juice, infusion of hay, and stagnant pools. His freezing was done by the winter's cold, and his figures were obtained by counting the contents of ten drops and taking an average. He found

[1] Macfadyen (Lancet, I, 1900, p. 849) has recently exposed cultures of Bacillus typhi, Bacillus coli communis, Bacillus diphtheriæ, Spirillum choleræ asiaticæ, Bacillus proteus vulgaris, Bacillus acidi lactici, Bacillus anthracis (spore bearing), Staphylococcus pyogenes aureus, Bacillus phosphorescens, and Photobacterium balticum in solid and liquid cultures to the temperature of liquid air (−182° C. to −190° C.), for twenty hours without sterilization and without impairing the properties of the organisms in any degree.

Macfadyen and Rowland (Lancet, Vol. I, 1900, p. 1130) treated the same organisms in broth emulsions in fine quill tubes with liquid air for seven days with the same results, except that a slightly delayed growth was noticed in some instances.

in the upper part of the ice 16 bacteria; in the lower part, only partially frozen, 250; in the upper and lower parts of a duplicate unfrozen vessel of water, 160 and 170, respectively. He obtained similar results with three species of Infusoria, and concluded that 90 per cent of the organisms were removed by freezing. His experiments appear, however, to show crystallization effects principally. The first careful work on this subject was done by Fraenkel in Berlin.[81] He collected river water, and after planting samples, froze them artificially at −8° to −12° C., thawing after different periods. In two days 83 per cent of the water bacteria present were killed; in three days 99 per cent; in five days, 90 per cent; in six days, 80 per cent; in six days, in another case, 93 per cent; and in nine days, 99 per cent. The different samples evidently varied greatly. Fraenkel also analyzed the regular Berlin ice-supply, and got results ranging from 21 to 9700 bacteria per cubic centimeter. He concluded that the ice was highly polluted and should not be taken into the system. About the same time Wolffhuegel and Riedel[82] gave an account of some experiments in which flasks of tap-water were kept in the ice-chest without freezing, and showed the following reductions: after one day, from 148 germs per cubic centimeter to 126 and from 150 to 115; after two days, from 123 to 69 and from 158 to 101; after three days, from 123 to 29 and from 156 to 33.

In 1887 Dr. Prudden of New York published the most exhaustive review hitherto attempted of the subject of quantitative reduction, and the first in which specific pathogenic germs were used.[83] His tubes, in the experiments with the latter organisms, were inoculated from pure cultures and frozen at −10° to −1° C., and his results were as follows, the numbers in each case referring to bacteria per cubic centimeter: —

B. prodigiosus. In water, 6300; in ice after 4 days, 2970; after 37 days, 22; after 51 days, 0.

Proteus vulgaris. In water, 8320; in ice after 18 days, 88; 51 days, 0.

Staphylococcus pyogenes aureus. In water, innumerable; in ice after 18 days, 224,598; 20 days, 46,486; 54 days, 34,320; 66 days, 49,280.

Species unnamed. In water, innumerable; in ice after 4 days, 571,450; 11 days, 520,520; 51 days, 183,040; 65 days, 10,978; 77 days, 85,008.

Species unnamed. In water, 800,000; in ice after 7 days, 0.

B. typhi. In water, innumerable; in ice after 11 days, 1,019,403; 27 days, 336,457; 42 days, 89,796; 69 days, 24,276; 77 days, 72,930; 103 days, 7348.

Same. In water, 378,000; in ice after 12 hours, 164,780; after 3 days, 236,676; 5 days, 21,416; 8 days, 76,032.

Dr. Prudden then made certain experiments to determine the effect of alternate

freezing and thawing, and obtained the following results. The tubes were here immersed in ice and salt at −20° C.

B. TYPHI.

In water . . .	40,896		
Frozen 24 hours	29,780	Refrozen 3 times	90
" 3 days	1,800	" 5 "	0
" 4 "	950	" 6 "	0
" 5 "	2,490	" 6 "	0

B. PRODIGIOSUS.

In water . . .	339,516		
Frozen 24 hours	36,410	Refrozen once	2,570
" 30 "	41,580	" 2 times	275
" 48 "	14,440	" 3 "	15
" 96 "	4,850	" 4 "	0

STAPHYLOCOCCUS PYOGENES AUREUS.

In water . . .	111,782		
Frozen 15 minutes	52,500		
" 2 hours	21,300		
" 24 "	22,690	Refrozen once	13,495
" 48 "	6,460	" 3 times	110
" 96 "	6,155	" 4 "	0

Dr. Prudden found that, with fresh, active agar cultures of this staphylococcus 49,280 germs remained alive, out of innumerable germs originally present, after sixty days; when cultures from old and dried agar were used, 162,000 germs disappeared entirely after five days. He ultimately drew the following conclusions from these experiments with pathogenic germs: 1. Many bacteria are killed by freezing. 2. The vitality of the original culture affects the number so killed. 3. The number killed varies with the species. 4. The number killed increases as the time of freezing is prolonged. 5. The resistance to cold varies with the individual bacterium. 6. Alternate freezing and thawing is very generally fatal.

Dr. Prudden also froze natural waters with their native bacteria for varying periods, and obtained somewhat similar results. He analyzed 270 samples of New York ice, and found an average of 2033 bacteria per cubic centimeter. The numbers were highest in the upper layers of snow ice and bubbly ice, and in ice cut in the immediate vicinity of Albany, falling off rapidly in ice five or six miles down the river. He concluded that this highly polluted ice probably contained the germs of typhoid fever and should not be taken into the human body.

Later in the same year Bordoni-Uffreduzzi [84] published a paper in which he took issue with Prudden on several points. He contended that the changes of temperature in the latter's experiments were too abrupt, that the resistance of the germs worked with had been weakened by cultivation on artificial media, and that the effect had been abnormally severe on account of the small size of the tubes frozen. He himself analyzed the natural water in one of the municipal basins of Berlin, just before a frost, and then kept a large lump of the ice in a double-walled zinc chest, breaking off samples for analysis every month. He found that about 90 per cent of the bacteria were killed, and thought the duration of the freezing did not make any material difference. His results, of course, varied very widely on account of the unequal distribution of the bacteria in the ice.

Russell [85] a little later made similar experiments at Madison, Wisconsin, in which he found that the ice formed on Lake Mendota contained about 40 per cent of the germs present in the water itself. A report already cited [21] was made by the State Board of Health of Massachusetts in 1889 in which ice from fifty-eight sources was analyzed in comparison with the water on which it had formed. Averaging all results, there were 81 per cent as many bacteria present in the snow ice as in the water, 10 per cent in all the rest of the ice, and only 2 per cent in the clear ice. In the report of the Board for the next year,[86] Mr. Hiram F. Mills noted an isolated but significant experiment in which sterilized tap water was inoculated with the typhoid germ, kept in a bottle surrounded by ice and sampled at intervals. The results were as follows: —

Day	Number of Typhoid Bacilli.	Day	Number of Typhoid Bacilli.
1	6120	15	100
5	3100	20	17
10	490	25	0

Taken altogether, more exact studies confirm the rough estimate of Pengra that some 90 per cent of ordinary water bacteria are eliminated by the process of freezing. As to the percentage reduction of specific pathogenes and, in particular, of the typhoid bacillus, probably the only form of great practical importance, the evidence is very meagre. The only results hitherto, as far as we have been able to discover, which fix quantitatively the effect of cold on this organism, are the three experiments of Dr. Prudden and the single experiment of the biologists of the Massachusetts State Board of Health. These certainly appear to form a slender basis for conclusions relative to the importance of ice-supply as a possible source of typhoid fever.

III. EXPERIMENTS BY THE AUTHORS ON THE EFFECT OF COLD UPON THE BACILLI OF TYPHOID FEVER.

A. EXPERIMENTS ON THE PERCENTAGE REDUCTION OF TYPHOID FEVER BACILLI EFFECTED BY FREEZING FOR DIFFERENT PERIODS OF TIME.

Methods Employed.

The following investigation was undertaken in order to so extend and amplify the work of Prudden as to obtain some idea of the average fatality occurring among typhoid bacilli in ice, and of the special conditions which affect such fatality. Pure cultures alone were used, as it is obvious that figures, to be of much value, must be determined separately for each specific germ. Great pains were taken to preserve, as far as possible, the vigor of the culture used, and new cultures from recent post-mortem examinations were obtained at intervals during the work. Finally, a large number of determinations were made for each set of conditions, in order to obtain average results free from the errors which may beset any individual case.

Our experiments on the percentage reduction effected by freezing were carried on by freezing small tubes of infected water, as only in this way can the conditions of the experiment be rigidly controlled. Ordinary test-tubes, containing about 10 cubic centimeters of sterilized tap water, were inoculated from a two or three day bouillon culture, and duplicate samples were at once planted. The ten tubes of the set under experiment were then placed in a double-walled tin vessel in which they were to be frozen. The inner vessel was a cylinder about 8 inches deep, nearly filled with a mixture of equal parts of glycerine and 95 per cent alcohol; in this solution the tubes were immersed, being supported by a disc perforated with holes to receive them. The solution served to make the lowering of temperature equal and gradual, and also acted as an antiseptic when the tubes broke, which sometimes happened when they contained too much water, or when the temperature went down too rapidly. In the outer vessel, which was jacketed with felt, was placed cracked ice which reduced the temperature of the glycerine-alcohol mixture to about 10°—15° C. in from an hour to an hour and a half. The ice was then replaced by a mixture of ice and salt which completed the freezing

in a half or three-quarters of an hour more. The time occupied by the whole process of freezing is recorded in the tabulation of each experiment. The temperature, in the first set of experiments with "Race A," was observed by means of three mercury thermometers inserted in different parts of the liquid, and at the time when the tubes froze the thermometers registered 6°–7° below zero, C. In later experiments the temperature was observed by means of a minimum registering spirit thermometer fastened to the inside of the cover of the inner cylinder, which recorded the temperature of the air just above the liquid in which the tubes were immersed. Partly on this account and probably partly because of its greater quickness of response, this thermometer gave lower records than did the mercury instruments in the first experiments. The readings of the spirit thermometer are given in the tables for each set of tubes.

As soon as the tubes froze, they were removed from the freezer and either thawed at once or kept frozen in an ice-chest for a few hours, or placed in a cold-storage warehouse where they were kept for the longer periods at a temperature one or two degrees below zero, C. After the frozen condition had been maintained for the desired length of time, the contents of the tubes were thawed, shaken up, and sampled, again in duplicate. As a rule the samples taken from the thawed tubes were planted directly, while those made before freezing were diluted, one to ten, with sterilized water. All plates, for these quantitative determinations, were planted with common nutrient agar-agar, containing 1.25 per cent agar, 1.00 per cent Witte's peptone, and .25 per cent salt, and having an acidity equal to 1.50 per cent. As the counts to be made were chiefly comparative, agar was preferred to any other medium, on account of its freedom from liquefaction. The plates were allowed to develop at the room temperature except in certain special cases to be noted later. Those made from the unfrozen water showed their maximum growth in three days and were counted after that interval. Those made from the thawed ice, however, were found to develop more slowly; for them five days was generally found sufficient, although after the longer periods of freezing as much as ten days was sometimes allowed. The plates were finally counted with the aid of a hand lens.

In many of the sets of experiments a control tube was included, which was treated just like the others except that it was not inoculated. Each series of tubes includes two lots of eight or ten each, frozen on two different days.

The cultures were grown in bouillon (containing 1.00 per cent peptone, .25 per cent salt, and 1.00 per cent acid), and were changed twice or three times a week. In the earlier experiments the tubes were inoculated from a culture grown at the room

temperature, itself inoculated from one grown at 37.5° C. In the later work the cultures were all kept at the room temperature.

When experiments made on the culture obtained in November, 1898, gave results somewhat different from those given by the culture used in February, it was decided that still a third culture from a different source must be compared with the first two. The results showed that the descendants of these different stocks exhibited slight though constant and persistent differences in their reaction to cold. We have called the cultures derived from these original sources "Races," for physiological races they apparently must be considered.

The first culture used, Race A, was obtained from the Boston City Hospital as a forty-hour-old blood-serum culture on February 23, 1898. Unfortunately, the history and tests applied to this culture in the Hospital were not recorded, beyond the fact that it had been isolated from an autopsy about two weeks previously, by the usual differential methods.

Race B was obtained by the kindness of Dr. M. W. Richardson of the Massachusetts General Hospital in the middle of November, 1898, with the following history. It had been isolated from the spinal canal, in a case of typhoid meningitis. It gave typical reactions in media as follows: bouillon, very motile; litmus milk, no coagulum, slight acid production; sugar-agar, no gas; peptone solution, no indol; gelatine slant stab, typical growth, no liquefaction; arsenic bouillon (Thoinot), no growth; Capaldi-Proskauer sol. No. 1, no growth; potato, no visible growth; tube medium of His, clouding without gas production; typhoid serum, perfect reaction.

Race C was obtained, January 14, 1899, by the courtesy of Dr. Pratt of the Boston City Hospital. It had been isolated, December 30, from the peritoneal cavity in a case of peritonitis following typhoid fever. It gave typical growths on the ordinary media, gelatine, bouillon, and glycerin-agar; it was motile in the hanging drop; it gave no indol and no gas in glucose solution; it was decolorized by the Gram method and reacted to typhoid serum.

Race D was isolated in the laboratory of the City Hospital, March 26, 1899, from the urethra. It was identified by the same tests used for Race C.

Results Obtained.

The percentage reductions recorded in the subjoined tables (pp. 492–498), summarized in final form, are as follows: —

PERCENTAGE REDUCTION OBSERVED IN EXPERIMENTS ON THE VIABILITY OF TYPHOID BACILLI IN ICE.

	Race A.	B.	C.	D.
Frozen 15 minutes	59.4	13.8		
" 30 "	63.7			
" 1½ hours		32.2		
" 2 "	73.6			
" 3 "		41.4	99.5	74.8
" 6 "	77.8			97.0
" 12 "		38.6		84.4
" 15 "			98.0	
" 24 "		53.8	82.7	99.0
" 3 days		98.4	99.9	
" 7 "		93.3	99.5	
" 2 weeks	99.8	99.4	99.9	
" 4 "	99.8			
" 8 "	99.8			
" 12 "	99.8			

Conclusions.

1. Evidently we may reaffirm for the bacillus of typhoid fever the first of Prudden's conclusions as to the various pathogenes with which he worked, namely, that many bacteria are killed by freezing. After two weeks' exposure to the freezing temperature an average of considerably over 99 per cent of the germs perished. Of the 140 tubes inoculated with Races A, B, and C, and frozen for periods of two weeks and over, all but nine showed a reduction of over 99 per cent; and of the nine, all but one showed a reduction of 98 per cent or over. We may safely conclude that less than 1 per cent of the typhoid germs present in water can survive fourteen days of freezing.

2. During the first half-hour of freezing a heavy reduction takes place, amounting, perhaps, to 50 per cent. The tubes exposed for such short times to the unfavorable conditions exhibit a remarkable variability among themselves. In the same set one tube may show no reduction, while its neighbor is rendered almost sterile. Whether these differences are due to the varying physical conditions in the individual tubes, or to variations in the biological character of the loopful of bacteria used for inoculation, is uncertain. From the general harmony of the results obtained it appears that this factor of variability, whatever it may be, is practically eliminated by the averaging of 20 tubes.

After this brief period of sudden but uncertain reduction, the destruction of the germs proceeds pretty regularly as a function of the time. Although the different races vary, there is in each race a steadily increased reduction, with slight variations, as the time of freezing is prolonged. After 14 days, even with the most resistant

stock, Race B, the reduction was over 99 per cent. The reduction now proceeds, however, with increasing slowness; the two or three germs per thousand which have survived thus far appear to possess special powers of resistance. Even after 12 weeks few of the individual tubes were rendered sterile. These results appeared so remarkable that special experiments were conducted to test their accuracy, as it was felt that perhaps the few germs developing from the thawed ice might have been introduced from the air, as was obviously the case in some instances. Fifty tubes of Races B and C were therefore frozen for periods of a week and a month; plates were planted from them, with special precautions, and incubated at 37.5°; and the developing colonies were examined individually. The results, as the appended tables show (see p. 492), confirm those of the general investigation. Of the 20 tubes inoculated with Race B and frozen for a month, 10 were sterile; 9 gave one sterile plate, and one with one or two colonies of what proved to be extraneous germs; tube IV. alone gave, on one plate, 7 germs per cubic centimeter, which examination in the hanging drop, and growth on gelatine, and potato, in milk and glucose solution, showed to be the original typhoid culture. So of the 30 tubes of Race C frozen for a week, 17 were sterile; 9 showed contamination, one or two germs per plate; the other four showed 15, 4, 1, and 267 typhoid bacilli per cubic centimeter. These experiments confirm the results of those observers who froze typhoid cultures containing millions of germs without effecting sterilization.

3. Prudden's statement that the number of bacteria killed by freezing varies with the species may be extended. It is evident that within the species B. typhi abdominalis there are races, each having a power of resistance of its own, dependent upon its history within and without the body. A comparison of the tables for the shorter periods of freezing shows clearly that Race C succumbed with much greater readiness to the influence of cold than did Race B; while Races A and D occupied an intermediate position. These differences appear constant through the various sets, so that in each race the progressively increased reduction with more prolonged freezing follows a parallel course. The facts cannot, we think, be attributed to differences in the immediate environment of the germs; such differences do produce their effect, cultivation for a time on agar, for example, causing a decrease in resistance. The last sort of change is, however, temporary and may be quickly reversed by cultivation in bouillon; while the race differences were permanent during the period of experimentation. Correlated with them were certain minor characters; for instance, the weakest race, Race C, grew more slowly than either of the others, and took perceptibly longer to produce a definite clouding in a liquid medium.

RACE A. SERIES I.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
1	8750	21	99.8
2	910	4	99.5
3	4910	1	99.9+
4	1465	1	99.9
5	900	–	—
6	2475	4	99.8
7	1260	3	99.8
8	1360	10	99.2
9	1535	1	99.9+
10	1030	7	99.3
11	35210	0	100.0
12	22575	3	99.9+
13	53060	1	99.9+
14	8575	–	—
15	94580	–	—
16	116235	1	99.9+
17	140175	–	—
18	95725	4	99.9+
19	4602	3	99.9
20	229950	2	99.9+
Average			99.8

Tubes **1–10**, frozen March 2, 1898, in 1½ hours; thawed May 25, after *12 weeks*.

Tubes **11–20**, frozen March 4, 1898, in 2 hours; thawed May 27, after *12 weeks*.

RACE A. SERIES II.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
21	10655	17	99.8
22	7695	42	99.4
23	3170	2	99.9
24	4265	2	99.9
25	90825	1	99.9+
26	79625	2	99.9+
27	5920	6	99.9
28	275	1	99.6
29	5400	1	99.9+
30	2085	3	99.9
31	11480	2	99.9+
32	24637	12	99.9
33	214200	9	99.9+
34	2760	7	99.7
35	10430	113	98.9
36	32110	4	99.9+
37	12757	7	99.9
38	26547	4	99.9+
39	15155	8	99.9
40	19890	1	99.9+
Average			99.8

Tubes **21–30**, frozen March 7, 1898, in 2½ hours; thawed May 2, after *8 weeks*.

Tubes **31–40**, frozen March 12, 1898, in 2¼ hours; thawed May 7, after *8 weeks*.

RACE A. SERIES III.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
41	3730	6	99.8
42	7880	5	99.9
43	2810	6	99.8
44	710	7	99.0
45	4470	4	99.9
46	9626	3	99.9+
47	10482	2	99.9+
48	3035	12	99.6
49	2085	11	99.5
50	5710	5	99.9
61	136710	5	99.9+
62	41230	3	99.9+
63	82215	1	99.9+
64	26285	5	99.9
65	22225	1	99.9+
66	19145	3	99.9+
67	Control	Control	—
68	12320	2	99.9+
69	10850	4	99.9+
70	10920	3	99.9+
Average			99.8

Tubes **41–50**, frozen March 16, 1898, in 2 hours; thawed April 13, after *4 weeks*.

Tubes **61–70**, frozen March 19, 1898, in 1½ hours; thawed April 16, after *4 weeks*.

RACE A. SERIES IV.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
51	24640	25	99.9
52	49000	10	99.9+
53	48930	30	99.9
54	40450	60	99.8
55	29340	30	99.9
56	282240	65	99.9+
57	44380	110	99.7
58	132300	50	99.9+
59	24185	25	99.9
60	93555	75	99.9
71	55650	–	—
72	Control	Control	—
73	52395	35	99.9
74	9230	70	99.2
75	86870	60	99.9
76	46025	25	99.9
77	1740	25	98.6
78	41825	5	99.9+
79	33155	35	99.9
80	23250	30	99.9
Average			99.8

Tubes **51–60**, frozen March 18, 1898, in 1¼ hours; thawed April 1, after *2 weeks*.

Tubes **71–80**, frozen March 21, 1898, in 2½ hours; thawed April 4, after *2 weeks*.

RACE A. SERIES V.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
171	628425	48090	92.3
172	5355	2040	61.9
173	520380	8610	98.3
174	355950	122535	65.6
175	354690	36540	89.7
176	206010	19775	90.4
177	474390	4000	99.0
178	402020	—	—
191	3365	15	99.5
192	3300	40	98.8
193	103320	30	99.9+
194	133875	275	99.8
195	348655	315315	9.6
196	40	70	0.0
197	214200	350	99.8
198	169155	64575	61.8
Average			77.8

Tubes **171–178**, frozen May 9, 1898, in 2 hours; thawed same day, after *6 hours*.

Tubes **191–198**, frozen May 13, 1898, in 2⅓ hours; thawed same day, after *6 hours*.

RACE A. SERIES VI.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
151	40775	21980	46.1
152	39235	17080	56.5
153	45465	14770	67.5
154	26530	15190	42.7
155	36295	14385	60.4
156	10710	5110	52.3
157	23520	3800	83.8
158	127260	40005	68.6
181	300	15	95.0
182	51030	9660	81.1
183	13265	1410	89.4
184	20475	2955	85.6
185	14595	1145	92.2
186	23415	805	96.6
187	22365	2915	87.0
188	2260	—	—
Average			73.6

Tubes **151–158**, frozen April 30, 1898, in 2¼ hours; thawed, same day, after *2 hours*.

Tubes **181–188**, frozen May 11, 1898, in 2⅓ hours; thawed same day, after *2 hours*.

RACE A. SERIES VII.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
81	1820	990	45.9
82	2795	40	98.6
83	1265	25	98.1
84	820	0	100.0
85	355	15	95.8
86	430	15	96.5
87	2515	2075	17.5
88	1285	0	100.0
89	755	10	98.7
90	165	5	97.0
101	25970	11340	56.3
102	11665	8015	31.3
103	16955	4555	73.1
104	30730	26355	14.2
105	Control	Control	—
106	8750	6510	25.6
107	9205	5525	40.0
108	9345	3380	63.8
109	20090	11410	43.2
110	14315	9170	35.9
Average			63.7

Tubes **81–90**, frozen March 25, 1898, in 1⅔ hours; thawed same day, after *30 minutes*.

Tubes **101–110**, frozen April 9, 1898, in 2 hours; thawed same day, after *30 minutes*.

RACE A. SERIES VIII.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
121	500220	715	99.9
122	492345	252640	48.7
123	57420	27755	51.7
124	53795	705	98.7
125	Control	Control	—
126	5705	955	83.2
127	124110	7175	94.2
128	77490	9800	87.3
161	33810	17640	47.9
162	276900	275940	.3
163	349020	120960	65.3
164	246645	111930	54.6
165	120775	62050	48.6
166	472500	236880	49.9
167	756550	505575	33.2
168	170100	123795	27.2
Average			59.4

Tubes **121–128**, frozen April 23, 1898, in 1½ hours; thawed same day, after *15 minutes*.

Tubes **161–168**, frozen May 4, 1898, in 1½ hours; thawed same day, after *15 minutes*.

RACE B. SERIES I.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
71	37275	462	98.8
72	45990	27	99.9
73	41685	189	99.5
74	63210	382	99.4
75	26250	773	97.1
76	34230	599	98.3
77	18800	378	98.0
78	40110	467	98.8
79	42525	613	98.6
80	50295	47	99.9
81	144325	23	99.9+
82	108360	11	99.9+
83	123165	8	99.9+
84	89775	7	99.9+
85	83790	9	99.9+
86	58275	10	99.9+
87	104895	21	99.9+
88	83475	11	99.9+
89	187110	51	99.9+
90	56595	15	99.9+
Average			99.4

Tubes **71–80**, frozen December 16, 1898, in 1⅓ hours; thawed December 30, after *2 weeks*. Minimal temperature, (−14° C.).

Tubes **81–90**, frozen December 17, 1898 in 2 hours; thawed December 31, after *2 weeks*. Minimal temperature, (−8° C.).

RACE B. SERIES II.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
111	52605	3622	93.1
112	88200	1386	98.4
113	95235	4018	95.8
114	63065	1270	98.0
115	31080	1165	96.3
116	43470	1470	96.6
117	47040	896	98.1
118	37065	511	98.6
119	32890	441	98.7
120	54495	2935	94.6
121	10290	2373	76.9
122	54705	4106	92.5
123	69990	1466	97.9
124	21175	2993	85.9
125	45150	—	—
126	61005	4452	92.7
127	61950	3633	94.1
128	114030	17042	85.1
129	90090	8127	91.0
130	6650	805	87.9
Average			93.3

Tubes **111–120**, frozen December 23, 1898, in 2 hours; thawed December 30, after *1 week*. Minimal temperature, (−10° C.).

Tubes **121–130**, frozen December 24, 1898, in 1½ hours; thawed December 31, after *1 week*. Minimal temperature, (−12° C.).

RACE B. SERIES III.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
91	34965	180	99.5
92	25445	55	99.8
93	28560	60	99.8
94	29085	165	99.4
95	33810	365	98.9
96	32745	25	99.9
97	26880	5705	78.8
98	15855	15	99.9
99	22330	75	99.7
100	90300	30	99.9+
151	2560	2	99.9
152	1595	4	99.7
153	1555	—	—
154	—	—	—
155	225	1	99.6
156	1195	4	99.6
157	95	2	97.9
158	80	1	98.8
159	30	0	100.0
160	25	0	100.0
Average			98.4

Tubes **91–100**, frozen December 20, 1898, in 2 hours; thawed December 23, after *3 days*. Minimal temperature, (−12° C.).

Tubes **151–160**, frozen January 3, 1899, in 2 hours; thawed January 6, after *3 days*. Minimal temperature, (−12° C.).

RACE B. SERIES IV.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
41	70560	38535	45.4
42	52290	31605	39.5
43	38640	28665	25.8
44	48405	10589	78.1
45	71505	14458	79.8
46	44100	10822	75.4
47	63945	21641	66.2
48	28245	13541	52.1
49	91035	19845	78.3
50	27300	11340	58.3
51	14140	5740	59.4
52	37800	25830	31.7
53	29925	15995	46.6
54	14280	5810	59.3
55	39710	16870	57.5
56	27825	9486	65.9
57	13685	5390	60.6
58	12565	5565	55.7
59	32760	33075	0.0
60	24570	9345	62.0
Average			53.8

Tubes **41–50**, frozen December 1, 1898, in 2 hours; thawed December 2, after *24 hours*. Minimal temperature, (−7° C.).

Tubes **51–60**, frozen December 8, 1898, in 2¼ hours; thawed December 9, after *24 hours*. Minimal temperature, (−10° C.).

RACE B. SERIES V.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
61	30135	13510	55.2
62	23625	8505	64.0
63	19635	10430	46.9
64	13055	12600	3.4
65	21840.	10500	51.9
66	13685	6720	50.9
67	16800	10535	37.3
68	12075	8435	30.1
69	13230	11130	15.9
70	18025	12740	29.3
101	32865	18515	43.7
102	31710	37275	0.0
103	42525	5670	86.7
104	32865	36225	0.0
105	4585	65	98.5
106	22050	9380	57.5
107	5280	184590	0.0
108	5267	206010	0.0
109	15155	0	100.0
110	4585	107740	0.0
Average			38.6

Tubes **61–70**, frozen December 9, 1898, in 2½ hours; thawed December 10, after *12 hours*. Minimal temperature, (–6° C.).

Tubes **101–110**, frozen December 21, 1898, in 1½ hours; thawed December 22, after *12 hours*. Minimal temperature, (–8° C.).

RACE B. SERIES VI.

Number of Tube.	Average number Bacteria per c. c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
21	10	200	0.0
22	75	170	0.0
23	190	70	63.2
24	2695	3360	0.0
25	100	250	0.0
26	210	375	0.0
27	1605	1505	6.2
28	180	350	0.0
29	1875	1825	2.7
30	3400	620	81.8
31	22905	12040	47.4
32	32655	8295	74.6
33	18550	6300	66.1
34	22225	6125	72.4
35	13755	4165	69.7
36	15575	3972	74.5
37	15750	7490	52.4
38	15470	3920	74.7
39	19215	5705	70.3
40	9590	2610	72.8
Average			41.4

Tubes **21–30**, frozen November 28, 1898, in 1½ hours; thawed same day, after *3 hours*. Minimal temperature, (–8° C.).

Tubes **31–40**, frozen November 29, 1898, in 1½ hours; thawed same day, after *3 hours*. Minimal temperature, (–8° C.).

RACE B. SERIES VII.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
1	3515	2080	40.8
2	2180	3000	0.0
3	3535	2620	25.9
4	4455	3105	30.3
5	Control	Control	—
6	4300	4325	0.0
7	4975	3525	29.1
8	3405	3460	0.0
9	4305	5970	0.0
10	4615	3225	30.1
11	7960	6300	20.8
12	16380	14490	11.5
13	7560	6860	9.2
14	19460	21560	0.0
15	12215	10080	17.5
16	21700	15085	30.5
17	7665	8400	0.0
18	13300	11060	16.8
19	10920	11340	0.0
20	10360	14770	0.0
Average			13.8

Tubes **1–10**, frozen November 19, 1898, in 2¼ hours; thawed, same day, after *15 minutes*.

Tubes **11–20**, frozen November 21, 1898, in 1¼ hours; thawed, same day, after *15 minutes*.

RACE C. SERIES I.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
1	6580	–	—
2	13475	5	99.9+
3	4795	7	99.9
4	9310	4	99.9+
5	10005	5	99.9+
6	10885	2	99.9+
7	6230	102	98.4
8	5215	0	100.0
9	10325	–	—
10	11550	6	99.9+
21	120645	12	99.9+
22	142065	16	99.9+
23	16695	0	100.0
24	0	0	—
25	0	1	—
26	13755	12	99.9
27	378945	1	99.9+
28	101115	0	100.0
29	4370	2	99.9+
30	128520	88	99.9
Average			99.9

Tubes **1–10**, frozen January 16, 1899, in 1¾ hours; thawed January 30, after *2 weeks*. Minimal temperature, (–13° C.).

Tubes **21–30**, frozen January 18, 1899, in 1¾ hours; thawed February 1, after *2 weeks*. Minimal temperature, (–10° C.).

RACE C. SERIES II.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water	Thawed Ice.	
51	1920	2	99.9
52	2675	2	99.9
53	2200	1	99.9+
54	2510	3	99.9
55	2065	33	98.4
56	1605	10	99.4
57	1685	1	99.1
58	835	13	98.4
59	460	15	96.7
60	1820	–	—
71	6580	0	100.0
72	7700	10	99.9
73	2485	1	99.9+
74	6440	1	99.9+
75	5145	3	99.9
76	4130	1	99.9+
77	3920	1	99.9+
78	3080	4	99.9
79	3535	0	100.0
80	540	0	100.0
Average			99.5

Tubes **51–60**, frozen January 23, 1899, in 1½ hours; thawed January 30, after *1 week*. Minimal temperature, (–12° C.).

Tubes **71–80**, frozen January 25, 1899, in 1½ hours; thawed February 1, after *1 week*. Minimal temperature, (–14° C.).

RACE C. SERIES III.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
11	16765	9	99.9
12	17220	0	100.0
13	14315	2	99.9+
14	900	2	99.8
15	18270	3	99.9+
16	9170	1	99.9+
17	6930	0	100.0
18	7385	0	100.0
19	2925	0	100.0
20	9555	1	99.9+
41	83475	6	99.9+
42	83160	5	99.9+
43	64890	2	99.9+
44	66570	4	99.9+
45	11200	1	99.9+
46	21350	23	99.9
47	2030	3	99.9
48	700	1	99.9
49	185	2	98.9
50	1625	2	99.9
Average			99.9

Tubes **11–20**, frozen January 17, 1899, in 1⅓ hours; thawed January 20, after *3 days*. Minimal temperature, (–13° C.).

Tubes **41–50**, frozen January 20, in 2 hours; thawed January 23, after *3 days*. Minimal temperature, (–10° C.).

RACE C. SERIES IV.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
61	3335	5	99.9
62	3520	25	99.3
63	195	10	94.9
64	885	55	93.8
65	235	25	89.4
66	215	60	72.1
67	2105	10	99.5
68	555	20	96.4
69	40	20	50.0
70	500	15	97.0
81	1855	85	95.4
82	1830	20	98.9
83	260	55	78.8
84	935	35	96.3
85	110	95	13.6
86	3595	30	99.2
87	4480	35	99.2
88	315	70	77.8
89	50	40	20.0
90	0	40	—
Average			82.7

Tubes **61–70**, frozen January 24, 1899, in 1⅓ hours; thawed January 25, after *24 hours*. Minimal temperature, (–12° C.).

Tubes **81–90**, frozen January 26, 1899, in 1½ hours; thawed January 27, after *24 hours*. Minimal temperature, (–13° C.).

RACE C. SERIES V.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
91	10710	20	99.8
92	7280	75	99.0
93	9555	90	99.1
94	4645	5	99.9
95	7735	35	99.5
96	1570	355	77.4
97	1325	20	98.5
98	—	—	—
99	6440	590	90.8
100	13090	10	99.9
111	143640	5	99.9+
112	234360	105	99.9+
113	105525	10	99.9+
114	41265	135	99.7
115	11655	5	99.9+
116	36855	20	99.9
117	27195	40	99.9
118	119070	50	99.9+
119	45360	5	99.9+
120	15855	—	—
Average			98.0

Tubes **91–100**, frozen January 27, 1899, in 2 hours; thawed January 28, after *15 hours*. Minimal temperature, (–14° C.).

Tubes **111–120**, frozen February 3, 1899, in 2 hours; thawed February 4, after *15 hours*. Minimal temperature, (–15° C.).

RACE C. SERIES VI.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
31	350	10	97.1
32	270	0	100.0
33	185	0	100.0
34	90	5	94.5
35	5	0	100.0
36	0	–	—
37	5	0	100.0
38	20	0	100.0
39	5	0	100.0
40	0	0	—
101	172080	1	99.9+
102	61110	9	99.9+
103	56700	1	99.9+
104	40005	4	99.9+
105	16660	0	100.0
106	146475	1	99.9+
107	8855	1	99.9+
108	9345	12	99.9
109	6930	2	99.9+
110	5075	0	100.0
Average			99.5

Tubes **31–40**, frozen January 19, 1899, in 2 hours; thawed same day, after *3 hours*. Minimal temperature, (–8° C.).

Tubes **101–110**, frozen February 2, 1899, in 2 hours; thawed same day, after *3 hours*.

RACE D. SERIES I.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
1	5355	3080	42.5
2	5915	3265	44.8
3	6090	2465	59.5
4	5670	670	88.2
5	3010	1615	46.3
6	4410	780	82.3
7	3745	365	90.3
8	3290	1000	69.6
9	4375	480	89.0
10	6580	3640	44.7
11	—	—	—
12	2380	95	96.0
13	—	—	—
14	—	—	—
15	7210	65	99.1
16	1855	40	97.8
17	3675	90	97.5
18	—	—	—
19	—	—	—
20	—	—	—
Average			74.8

Tubes **1–10**, frozen April 27, 1899, in 2 hours; thawed same day, after *3 hours*. Minimal temperature, (–16° C.).

Tubes **11–20**, frozen April 28, 1899, in 2 hours; thawed same day, after *3 hours*. Minimal temperature, (–14° C.).

RACE D. SERIES II.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice	
31	52605	318	99.4
32	53235	5072	90.5
33	77175	52	99.9
34	5565	927	83.3
35	184275	7339	96.0
36	6580	420	93.6
37	1890	—	—
38	62055	6457	89.6
39	3255	87	97.3
40	6020	134	97.8
71	24360	2	99.9+
72	29505	2	99.9+
73	8925	22	99.8
74	2430	0	100.0
75	12810	4	99.9+
76	24355	3	99.9+
77	9450	210	97.8
78	2065	1	99.9+
79	3160	1	99.9+
80	2185	1	99.9+
Average			97.0

Tubes **31–40**, frozen May 1, 1899, in 2 hours; thawed same day, after *6 hours*. Minimal temperature, (–10° C.).

Tubes **71–80**, frozen May 8, 1899, in 2 hours; thawed same day, after *6 hours*. Minimal temperature, (–16° C.)

RACE D. SERIES III.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
21	3990	15	99.6
22	3675	20	99.5
23	670	15	97.8
24	180	100	44.4
25	595	45	92.4
26	2275	15	99.3
27	180	20	88.9
28	140	25	83.6
29	25	25	0.0
30	240	0	100.0
41	515	33	93.6
42	1575	152	90.3
43	495	39	92.2
44	—	—	—
45	1855	88	95.3
46	2625	409	84.4
47	—	—	—
48	—	—	—
49	7175	511	92.9
50	1025	192	81.3
Average			84.4

Tubes **21–30**, frozen April 28, 1899, in 2 hours; thawed April 29, after *12 hours*. Minimal temperature, (–11° C.).

Tubes **41–50**, frozen May 1, 1899, in 2 hours; thawed May 2, after *12 hours*. Minimal temperature, (–10° C.).

RACE D. SERIES IV.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
51	33915	—	—
52	24570	106	99.6
53	60795	33	99.9
54	15960	35	99.8
55	24805	89	99.6
56	8820	103	98.8
57	6860	6	99.9
58	8960	55	99.4
59	2660	29	98.9
60	130410	199	99.8
61	21735	32	99.9
62	3200	71	97.8
63	5215	5	99.9
64	6160	63	99.0
65	955	10	99.0
66	2085	40	98.1
67	10885	65	99.4
68	250	3	98.8
69	790	34	95.7
70	1150	23	98.0
Average			99.0

Tubes **51–60**, frozen May 2, 1899, in 2 hours; thawed May 3, after *24 hours*. Minimal temperature, (−13° C.).
Tubes **61–70**, frozen May 3, in 2 hours; thawed May 4, after *24 hours*. Minimal temperature, (−12° C).

RACE B. SPECIAL SERIES.

Number of Tube.	Number Colonies per c.c. Unfrozen Water.		Number Colonies per c.c. Thawed Ice.		
I	56070	83790	0	0	
II	55440	53550	0	0	
III	62370	61110	1	0	
IV	19320	21210	0	7	*
V	42210	29190	0	0	
VI	28980	30030	0	0	
VII	28770	18060	1	0	
VIII	23730	33390	0	1	
IX	46410	42630	1	2	
X	—	7420	0	1	
XI	13720	12180	0	0	
XII	22050	28980	1	0	
XIII	11830	7700	0	0	
XIV	7980	7560	0	0	
XV	7070	6020	1	0	
XVI	6020	4690	0	0	
XVII	5810	4690	0	0	
XVIII	1840	1850	0	1	
XIX	1260	1510	1	0	
XX	3430	4200	0	0	

Tubes **I–X**, frozen February 10, 1899, in 4 hours; thawed March 10, after *4 weeks*. Minimal temperature, (−10° C.).
Tubes **XI–XX**, frozen February 15, in 1½ hours; thawed March 15, after *4 weeks*. Minimal temperature, (−10° C.).

* Colonies in ice of Tube IV proved to be typhoid. Colonies in ice in tubes not starred proved to be contaminations.

RACE C. SPECIAL SERIES.

Number of Tube.	Number Colonies per c.c. Unfrozen Water.		Number Colonies per c.c. Thawed Ice.			Number of Tube.	Number Colonies per c.c. Unfrozen Water.		Number Colonies per c.c. Thawed Ice.		
I	4690	5390	0	0		**XVI**	0	9	0	0	
II	6440	5390	0	1		**XVII**	21840	17640	0	0	
III	7140	5320	0	1		**XVIII**	9520	14070	0	0	
IV	9870	12880	18	12	*	**XIX**	15960	8680	0	0	
V	5560	7210	3	4	*	**XX**	7910	16170	0	0	
VI	10080	11060	0	1		**XXI**	25410	30450	3	1	
VII	4060	3710	0	1		**XXII**	34020	31920	0	–	
VIII	4480	3990	0	2		**XXIII**	3	0	0	0	
IX	1	1	0	0		**XXIV**	20790	17640	0	–	
X	1	2	1	–		**XXV**	107730	103950	272	262	*
XI	147420	148050	0	–		**XXVI**	380	560	0	1	
XII	2	0	0	0		**XXVII**	0	0	0	0	
XIII	63630	71190	0	0		**XXVIII**	330	210	0	1	*
XIV	57960	48510	0	0		**XXIX**	150	280	0	0	
XV	87570	86940	1	0		**XXX**	1330	1440	0	0	

Tubes **I–X**, frozen February 17, 1899, in 3 hours; **XI–XX**, February 23, in 2 hours; **XXI–XXX**, March 3, in 1¾ hours; thawed after *1 week* in each case. Minimal temperature, −10° C.

* Colonies in ice proved to be typhoid. Colonies in ice in tubes not starred proved to be contaminations.

B. EXPERIMENTS ON THE EFFECT OF ALTERNATE FREEZING AND THAWING UPON THE BACILLI OF TYPHOID FEVER.

Dr. Prudden, as we have seen, considered intermittent more fatal than uninterrupted freezing, and, indeed, succeeded in one case in entirely sterilizing a tube inoculated with B. typhi by this method. Our four series of experiments on this subject were conducted by freezing tubes in the freezer as described in the previous section. The tubes of Series I, Race A, were frozen daily for five days and allowed to thaw each time after about eighteen hours, samples being planted after each thawing. Those of Series I, Race B, were frozen three times, on alternate days, remaining frozen for twenty-four hours each time and kept below 2° for the rest of the time. The two series in Race D were treated like the tubes frozen for three hours and six hours in the last section, except that instead of remaining frozen they were thawed and refrozen once and twice respectively during that time.

The results of these experiments with the results of simple freezing directly comparable are as follows: —

RACE A.

	Reduction.
Frozen once in one day	96.1
Frozen twice in two days	98.9
Frozen three times in three days	99.5
Frozen four times in four days	99.8

RACE B.

Kept frozen for three days (see previous section, Race B, Series III)	98.4
Frozen twice in four days	99.6
Kept frozen for seven days (see previous section, Race B, Series II)	93.3
Frozen three times in six days	99.8

RACE D.

Kept frozen for three hours (see previous section, Race D, Series I)	74.8
Refrozen once in three hours	97.4
Kept frozen for six hours (see previous section, Race D, Series II)	97.0
Refrozen twice in six hours	99.5

CONCLUSION. Thawing and refreezing are somewhat more fatal than simple freezing in its effect on the typhoid bacillus. Four successive freezings and thawings do not, however, suffice to kill off the most resistant bacilli.

RACE A. SERIES I.

Number of Tube.	Average before Freezing.	After One Freezing.		After Two Freezings.		After Three Freezings.		After Four Freezings.	
		Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.
91	49910	—	—	—	—	—	—	—	—
92	22785	175	99.3	6	99.9	1	99.9	3	99.9
94	348390	26320	92.5	2495	99.3	644	99.9	147	99.9
95	308385	1735	99.5	171	99.9	59	99.9	2	99.9
96	167580	535	99.7	6	99.9	2	99.9	2	99.9
97	277515	40	99.9	1	99.9	3	99.9	2	99.9
98	50820	745	98.5	290	99.4	200	99.6	120	99.8
99	600	180	70.0	4	99.3	1	99.8	—	—
100	34090	190	99.4	55	99.9	16	99.9	8	99.9
111	76895	380	99.5	80	99.9	22	99.9	20	99.9
112	23875	685	97.1	355	98.5	33	99.9	13	99.9
113	29750	—	—	—	—	—	—	—	—
114	38290	265	99.3	40	99.9	3	99.9	0	100.0
115	31500	1785	94.3	1232	96.1	416	98.7	427	98.6
116	46585	75	99.8	3	99.9	23	99.9	3	99.9
117	21	17	—	15	—	2	—	2	—
118	48335	4450	90.8	3895	91.9	2440	95.0	—	—
119	38430	155	99.6	11	99.9	2	99.9	2	99.9
120	25200	215	99.1	14	99.9	2	99.9	3	99.9
	Averages		96.1		98.9		99.5		99.8

Tubes **91–100**, frozen March 28, 1898; thawed and sampled and refrozen, on each of the four days succeeding. Tubes remained frozen 18 hours each time.

Tubes **111–120**, treated in same manner week of April 11, 1898.

RACE B. SERIES I.

Number of Tube.	Average before Freezing.	After One Freezing.		After Two Freezings.		After Three Freezings.	
		Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.
1	46950	420	99.1	67	99.9	38	99.9
2	22260	155	99.3	24	99.9	23	99.9
3	12810	315	97.5	21	99.8	19	99.9
4	12145	215	98.2	52	99.6	29	99.8
5	10640	20	99.8	10	99.9	7	99.9
6	8715	120	98.6	10	99.9	5	99.9
7	7945	80	99.0	13	99.8	9	99.9
8	4190	65	98.4	13	99.7	7	99.8
9	2520	85	96.6	9	99.6	4	99.8
10	2450	95	96.1	7	99.7	1	99.9+
11	115290	775	99.3	280	99.8	161	99.9
12	142695	1980	98.6	1008	99.3	356	99.8
13	183385	315	99.8	96	99.9	85	99.9+
14	74970	1140	98.5	595	99.2	354	99.5
15	138915	480	99.7	276	99.8	116	99.9
16	227745	11865	94.8	5733	97.5	458	99.8
17	104265	670	99.4	198	99.8	129	99.9
18	107730	1250	98.8	403	99.6	269	99.8
19	163485	650	99.6	139	99.9	65	99.9+
20	120015	390	99.7	171	99.9	75	99.9
	Averages		98.5		99.6		99.8

Tubes **1–10**, frozen April 10, 1899; kept frozen for 24 hours, and below 2° for 24 hours more; refrozen April 12 and April 14. Samples planted before each freezing and April 15.

Tubes **11–20**, treated in same way, April 17, and following days.

RACE D. SERIES I.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
101	78435	147	99.8
102	76230	451	99.4
103	1765	23	98.7
104	2730	47	98.3
105	275	15	94.5
106	7735	26	99.7
107	1120	49	95.6
108	11690	134	98.9
109	6895	235	96.6
110	—	—	—
131	—	—	—
132	—	—	—
133	3500	175	95.0
134	—	—	—
135	29190	77	99.7
136	20160	371	98.2
137	6055	192	96.8
138	5710	388	93.2
139	—	—	—
140	3885	144	96.3
Average			97.4

Tubes **101–110**, frozen May 5, 1899; thawed and refrozen in *3 hours*. Minimal temperature, (−13° C.).
Tubes **131–140**, frozen May 13, 1899; thawed and refrozen in *3 hours*. Minimal temperature, (−19° C.).

RACE D. SERIES II.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
111	34020	71	99.8
112	59090	152	99.7
113	13230	7	99.9
114	32525	274	99.2
115	1545	9	99.4
116	4270	29	99.3
117	2095	1	99.9+
118	6685	5	99.9
119	5250	68	98.7
120	13685	210	98.5
121	—	—	—
122	—	—	—
123	203175	3	99.9+
124	40005	1	99.9+
125	56070	2	99.9+
126	170100	297	99.8
127	—	—	—
128	—	—	—
129	—	—	—
130	925	1	99.9
Average			99.5

Tubes **111–120**, frozen May 9, 1899; thawed and refrozen twice in next *6 hours*. Minimal temperature, (−14° C.).
Tubes **121–130**, frozen May 10, 1899; thawed and refrozen twice in next *6 hours*. Minimal temperature, (−10° C.).

C. EXPERIMENTS ON THE EFFECT OF TEMPERATURES SLIGHTLY ABOVE THE FREEZING-POINT UPON TYPHOID BACILLI IN WATER.

In these experiments sterilized test tubes were inoculated with pure cultures as in all the preceding work. Afterward they were treated in one of three ways, — either placed in an incubator at the room temperature, 20° C., or in an ice-chest ranging from 8°–12°, or cooled in the freezer to a point just above freezing. This last was effected by filling the outer chamber with ice without salt.

In the three sets of tubes treated by the last method at 1°, the duration of exposure and the reduction were as follows: Race A in two hours was reduced 47.8 per cent; Race B in one and one-half hours was reduced 32.9 per cent; Race C in three hours was reduced 80.1 per cent. The reductions for the same races actually frozen for the nearest corresponding periods, were 73.6 per cent, 41.4 per cent, and 99.5 per cent, respectively. Each race maintains its relative position of resistance. The reduction in the chilled water is very nearly as great as in the ice; and the difference is only what the temperature difference might be expected to produce. Evidently there

is nothing mysterious about the act of freezing, no mechanical crushing of bacteria; the process of destruction is continuous above and below the freezing-point, depending upon the two main factors of time and temperature.

Series II and III of Races B and C cover longer periods of time and higher temperatures. Half of the tubes in each series were kept at 10° and half at 20°, but no marked differences appeared as the result of these two modes of treatment, and the two sets are averaged together in each series. The tubes were kept in these experiments for two weeks, one-half of them being sampled on the second and the seventh day, the others on the third day and the fourteenth. The tubes were, of course, protected from the action of light.

	Race B.		Race C.	
	Reduction per cent.		Reduction per cent.	
Time.	Series II.	Series III.	Series II.	Series III.
1 day	92.2	78.7	88.4	71.0
3 days	99.3	86.7	90.4	83.3
7 days	99.9	99.3	94.1	89.6
14 days	99.9	98.8	99.9	99.9

It will be noted that with each race the second series shows a greater reduction than the third. The explanation for this lies in the fact that these experiments were carried on some time after the regular experiments on freezing their respective races. During the intervening period the germs had been grown on agar, and the first new series of experiments with each race showed an extraordinary reduction, over 99 per cent in a day, etc. The results of this series have not been tabulated. The second series of each race, Series II above, showed more moderate, but still high reductions; while by the time the third series was inoculated, a week later, the cultures, by cultivation in bouillon, had regained their normal condition.

The tubes inoculated with Race D were kept for twenty-four hours only, samples being planted after 3, 6, 12, and 24 hours. Series I was kept at 20° and Series II at 10°.

	After 3 hours.	6 hours.	12 hours.	24 hours.
Series I (20°)	70.8	72.6	85.7	88.4
Series II (10°)	63.1	74.0	87.4	95.5

Conclusions. From these experiments it appears that typhoid fever bacilli behave in water much as they do in ice. A large proportion of them are killed by a few minutes' exposure to the unfavorable conditions; during the next few hours the reduction proceeds *pari passu* with the duration of the experiment; while a few germs persist for some time.

The results differ from those obtained by actual freezing in two respects. We have seen that freezing for short periods produced varying and uncertain results, while ice over twenty-four hours old showed a constant reduction of over 90 per cent. The tubes of water which were not frozen remained subject to this uncertainty for a much longer period. Inspection of the tables will show that individual tubes contained sometimes half of their original germ content after a week, or four-fifths of it after three days. On the other hand, complete sterilization ensued more often than in the frozen tubes.

A second characteristic of the viability of the germs in water is the fact, closely allied to the first, that an increase seems sometimes to occur. The successive samplings of the same tube show in certain instances a slight multiplication.

The reduction in water at 10° does not seem to be any greater than at 20°.

RACE A. SERIES I.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
131	140490	51450	63.4
132	505	10	98.0
133	10640	11410	0.0
134	12390	6965	43.8
135	31465	165	99.5
136	273105	87885	67.8
137	17745	9870	44.4
138	112770	63000	44.1
141	254205	105840	58.4
142	157815	92610	41.3
143	72135	42490	41.1
145	302715	302715	0.0
146	141750	45990	67.6
147	17360	21735	0.0
Average			47.8

Tubes **131–138**, cooled down to 1° C. in 1¼ hours, April 25, 1898. Kept at that temperature for ½ hour more.

Tubes **141–147**, cooled down to 1° C. in 1½ hours, April 29, 1898. Kept at that temperature for ½ hour more.

RACE B. SERIES I.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
131	1946	1690	13.2
132	1610	1001	37.8
133	1848	1165	37.0
134	1571	1183	24.7
135	1379	1155	16.1
136	1291	1505	0.0
137	1232	1438	0.0
138	874	962	0.0
139	892	1473	0.0
140	1022	1051	0.0
141	4095	5	99.9
142	260	50	80.8
143	205	90	56.1
144	270	55	79.6
145	40	5	87.5
146	215	145	32.6
147	290	275	5.2
148	225	140	37.8
149	215	120	44.2
150	80	75	6.3
Average			32.9

Tubes **131–140**, cooled down to 0°, without freezing, and kept at that temperature for 1½ hours. Date, December 29, 1898.

Tubes **141–150**, cooled down to 0°, without freezing, and kept at that temperature for 1½ hours. Date, January 2, 1899.

RACE C. SERIES I.

Number of Tube.	Average Number Bacteria per c.c.		Reduction per cent.
	Unfrozen Water.	Thawed Ice.	
121	55125	14910	73.0
122	244755	217350	11.2
123	66465	11045	83.4
124	62685	59010	5.9
125	211050	32760	84.5
126	269955	75915	71.9
127	103005	7385	92.8
128	105840	8085	92.4
129	67725	20685	69.5
130	23100	4725	79.5
131	139860	9660	93.1
132	76545	3675	95.2
133	58275	6580	88.7
134	219135	23205	89.4
135	82530	3675	95.6
136	84105	3150	96.3
137	1290	0	100.0
138	38850	2105	94.6
139	30030	860	97.1
140	5530	640	88.4
Average			80.1

Tubes **121–130**, cooled down to 0° in ½ hour, February 6, 1899; kept at that temperature (not frozen) for three hours.

Tubes **131–140**, cooled down to 0° in ½ hour, February 7, 1899; kept at that temperature (not frozen) for 3 hours.

RACE B. SERIES II.

Number of Tube.	Average after Inoculation.	After One Day.		After Three Days.		After Seven Days.		After Fourteen Days.	
		Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.
201	232155	20	99.9+			2	99.9+		
202	93870	6755	92.8			0	100.0		
203	104895	4515	95.7			0	100.0		
204	9345	1180	87.4			0	100.0		
205	72135	60	99.9			1	99.9+		
206	51660			0	100.0			0	100.0
207	56070			1837	96.3			3	99.9+
208	1515			0	100.0			0	100.0
209	216405			2972	98.8			21	99.9+
210	Control			Control				Control	
211	11025	0	100.0			1	99.9+		
212	17885	5075	71.6			51	99.7		
213	20790	90	99.6			0	100.0		
214	10420	30	99.7			0	100.0		
215	81270	2020	75.2			73	99.9		
216	10640			1	99.9+			1	99.9+
217	825			0	100.0			8	99.0
218	170			1	99.4			0	100.0
219	22330			5	99.9+			0	100.0
220	74340			623	99.2			3	99.9+
	Averages		92.2		99.3		99.9		99.9

Tubes 201–210, inoculated March 17, 1899; kept in ice-chest at about 10° C.
Tubes 210–220, inoculated March 17, 1899; kept in room at about 20° C.

RACE B. SERIES III.

Number of Tube.	Average after Inoculation.	After One Day.		After Three Days.		After Seven Days.		After Fourteen Days.	
		Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.
221	113400	21630	80.8			2467	97.8		
222	74340	385	99.5			3	99.9+		
223	74340	13405	82.0			1043	98.6		
224	48510	7840	83.8			1043	97.8		
225	137025	2425	98.2			0	100.0		
226	17535			1389	92.1			0	100.0
227	103635			40446	61.0			6835	93.4
228	74655			27405	63.3			1603	97.9
229	25200			2520	90.0			28	99.9
230	85050			7465	91.2			112	99.9
231	34650	5355				30	99.9		
232	18795	6930				5	99.9+		
233	7320	1205				39	99.5		
234	5145	3420				9	99.8		
235	5565	1195				16	99.7		
236	5075		84.5	22	99.6			1	99.9+
237	10535		63.1	312	97.0			2	99.9+
238	4340		83.5	28	99.3			3	99.9
239	17955		33.5	4284	76.1			486	97.3
240	6090		78.5	173	97.2			3	99.9+
	Averages		78.7		86.7		99.3		98.8

Tubes 221–230, inoculated March 24, 1899; kept in ice-chest at about 10° C.
Tubes 231–240, inoculated March 24, 1899; kept in room at about 20° C.

RACE C. SERIES II.

Number of Tube.	Average after Inoculation.	After One Day.		After Three Days.		After Seven Days.		After Fourteen Days.	
		Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction. per cent.	Average.	Reduction per cent.
141	68985	1596	97.7			30	99.9+		
142	81270	10206	87.4			1043	98.7		
143	143640	1141	99.2			56	99.9+		
144	198450	107730	45.7			125956	36.5		
145	132300	1883	98.6			38	99.9+		
146	198450			13041	93.4			196	99.9
147	210735			2142	99.0			30	99.9+
148	80325			238	99.9+			2	99.9+
149	82215			479	99.9			2	99.9+
150	79065			228	99.9+			0	100.0
151	51345	1176	97.7			2	99.9+		
152	66780	14238	78.7			501	99.2		
153	349650	11970	96.9			128	99.9+		
154	73395	7	99.9+			0	100.0		
155	230580	40761	82.3			8347	96.4		
156	168210			135229	19.6			54	99.9+
157	41265			1400	96.6			22	99.9
158	17395			16	99.9			1	99.9+
159	83790			3402	95.9			5	99.9+
160	120015			42	99.9+			3	99.9+
	Averages		88.4		90.4		94.1		99.9

Tubes **141–150**, inoculated March 20, 1899; kept in ice-chest at 10°.
Tubes **151–160**, inoculated March 20, 1899; kept in room at about 20° C.

RACE C. SERIES III.

Number of Tube.	Average after Inoculation.	After One Day.		After Three Days.		After Seven Days.		After Fourteen Days.	
		Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.
161	10535	15	99.9			1	99.9+		
162	26985	5	99.9+			1	99.9+		
163	44205	30660	30.6			242	99.5		
164	5705	30	99.4			0	100.0		
165	340	230	32.4						
166	41685			7497	82.0			119	99.7
167	1080			45	95.8			1	99.9
168	24465			2709	88.9.			14	99.9
169	15330			15876	0.0				
170	Control			Control				Control	
171	420	40	90.5			0	100.0		
172	1205	805	33.2			1	99.9		
173	305	0	100.0			1	99.7		
174	19740	9275	53.0			609	96.9		
175	1065	310	70.9			2	99.8		
176	3255			46	98.6			1	99.9+
177	4105			271	93.4			2	99.9+
178	1725			1	99.9			0	100.0
179	620			0	100.0			2	99.7
180	17850			1596	91.1			48	99.7
	Averages		71.0		83.3		89.6		99.9

Tubes **161–170**, inoculated March 27, 1899; kept in ice-chest at 10°.
Tubes **171–180**, inoculated March 27, 1899; kept in room at about 20° C.

RACE D. SERIES I.

Number of Tube.	Average after Inoculation.	After Three Hours.		After Six Hours.		After Twelve Hours.		After Twenty-four Hours.	
		Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.
141	2590	30	98.8	0	100.0	5	99.8	5	99.8
142	5670	25	99.5	20	99.6	0	100.0	5	99.9
143	2315	5	99.8	225	90.3	0	100.0	10	99.6
144	3185	140	95.6	90	97.2	20	99.4	0	100.0
145	15	10	33.3	15	0.0	10	33.3	—	—
146	340	70	79.4	60	82.4	10	97.1	25	92.6
147	745	30	96.0	210	71.8	5	99.3	10	98.7
148	50	35	30.0	0	100.0	0	100.0	20	60.0
149	45	30	33.3	50	0.0	0	100.0	5	88.9
150	230	40	82.6	5	97.8	5	97.8	5	97.8
151	52920	22890	56.7	18690	64.7	7969	84.9	770	98.5
152	8680	6300	27.4	3780	56.5	1704	80.1	483	94.4
153	68670	28350	58.7	26885	60.8	21168	69.2	20097	70.7
154	43155	715	98.3	385	99.1	252	99.4	119	99.7
155	6650	1025	84.6	380	94.3	273	95.9	158	97.6
156	41895	7000	83.3	7105	83.0	5323	87.3	4410	89.5
157	74025	12355	83.3	14700	80.1	11056	85.1	6647	91.0
158	53235	7980	85.0	13125	75.3	14647	72.5	5386	89.9
159	23205	6090	73.8	9695	58.2	8064	65.2	10206	56.0
160	3255	2745	16.0	1925	40.9	1669	48.7	1480	54.5
	Averages		70.8		72.6		85.7		88.4

Tubes **141–150**, inoculated May 11, 1899. Kept at room temperature.
Tubes **151–160**, inoculated May 15, 1899. Kept at room temperature.

RACE D. SERIES II.

Number of Tube.	Average after Inoculation.	After Three Hours.		After Six Hours.		After Twelve Hours.		After Twenty-four Hours.	
		Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.	Average.	Reduction per cent.
161	27615	7455	73.9	5810	79.0	—	—	6268	77.3
162	291060	47145	83.8	21000	92.8	—	—	3895	98.7
163	200340	11235	43.9	205	99.9	—	—	1	99.9+
164	208530	24045	88.5	10500	95.0	—	—	5261	97.5
165	178605	1860	99.0	355	99.8	—	—	21	99.9+
166	54810	7000	87.2	—	—	—	—	—	—
167	106785	11655	89.1	2275	97.9	—	—	1543	86.4
168	212625	4130	98.1	1385	99.4	—	—	2001	99.1
169	25095	1960	92.2	745	97.1	—	—	529	97.9
170	2660	145	94.5	35	98.7	—	—	129	95.2
171	236250	95445	59.6	78435	66.8	25578	89.2	3643	98.5
172	240975	120330	50.1	144585	40.0	45360	81.3	20128	91.8
173	199395	98595	50.6	41840	79.0	16065	91.9	196	99.9
174	299565	150255	49.8	156200	47.8	29578	90.1	5691	98.1
175	179550	104895	41.6	54180	69.8	9670	94.4	2992	98.3
176	107730	64260	40.4	72265	32.9	17010	84.2	2396	97.8
177	133235	69930	47.5	81900	38.5	17671	86.7	7140	94.6
178	141750	89515	36.8	35700	74.8	20790	85.3	1101	99.2
179	211680	226800	0.0	158760	25.0	56891	73.1	31468	85.1
180	47565	30765	35.3	8610	71.9	1141	97.6	288	99.4
	Averages		63.1		74.0		87.4		95.5

Tubes **161–170**, inoculated May 17, 1899. Kept in ice-chest at 10°.
Tubes **171–180**, inoculated May 19, 1899. Kept in ice-chest at 10°.

D. EXPERIMENTS ON THE VIABILITY OF TYPHOID FEVER BACILLI IN EARTH AT VARIOUS TEMPERATURES.

These experiments were carried on in order to compare the conditions affecting a reduction of the number of typhoid bacilli in soil, with those operating on them in water and ice. The general method pursued was the same, the inoculation of numerous small portions of a sterile medium with a pure culture of the micro-organism. In each series of experiments about one hundred grains of sifted clayey soil were sterilized by baking for sixteen hours, on two successive days. The whole of the earth was then inoculated by mixing with it a bouillon culture two or three days old, of B. typhi, Race B; and an even distribution was accomplished by stirring and kneading with a spatula. The earth, having been dried by the previous baking, absorbed the bouillon culture without becoming visibly damp. Fifty portions of the inoculated earth of one gram each were then weighed out and placed in fifty sterile empty test-tubes. Of these fifty portions, ten were at once mixed with sterile water and two check plates made from each flask. The remaining forty tubes were carried to the cold storage warehouse or kept at the room temperature, as the case might be, in either condition being protected from the action of light. After one day, three days, one week, and two weeks, ten tubes were removed and planted. In every case the entire gram of earth was mixed with ten, one hundred or nine hundred cubic centimeters of sterile water; and two check plates were made from the dilution.

The inoculation, weighing and tubing of the earth, were conducted in a glass chamber some three feet square, with a sliding door raised only sufficiently to admit the arms of the operator. Control plates were made from four portions of the earth before inoculation, the portions of a gram apiece being tubed and planted exactly like the regular tubes. The following were the results per gram: —

Colonies per Gram.

1		2		3		4	
14	1	0	3	0	0	360	0

The tubes of the first three series were kept at the cold storage warehouse during the period of the experiment, at 0° C. Those of the fourth series were kept at the room temperature. The summarized average results of these four series are as follows: —

TYPHOID BACILLI IN EARTH. AVERAGE NUMBER PER GRAM.

		After Inoculation.	After 1 day.	3 days.	7 days.	14 days.
Series I	0°	180776	4635	705	25	9
II	0°	4846855	95017	1395	525	588
III	0°	7778595	324588	4656	1304	1160
IV	20°	4673683	2565	450	95	92

Two more series of experiments with earth were carried out to throw light on the part played by dryness in the reduction manifest in the first experiments. In these latter researches the sets of fifty tubes were inoculated just as before, and ten of them were planted at once. The remaining forty were divided into two portions. The gram of earth in each of twenty of the tubes was moistened by the addition of about one-third cubic centimeter of sterilized tap water; while the earth in the other twenty tubes was left in its comparatively dry condition. The tubes were all kept at the room temperature. Thus a comparison may be drawn as to the viability of the germ in damp and in dry earth. The results were as follows: —

TYPHOID BACILLI IN DRY AND DAMP EARTH. AVERAGE NUMBER PER GRAM.

	After Inoculation.		After 1 day.	3 days.	7 days.	14 days.
Series V	939115	Dry	2070	50	2	47
		Damp	225	7010	1295	8
VI	1198260	Dry	566	71	12	4
		Damp	1699110	—	—	29587

CONCLUSIONS. 1. The typhoid bacilli in dry earth behave just as in water and in ice. They die out, rapidly at first, and their numbers are progressively reduced as the treatment is prolonged. A fraction of one per cent persists for some time.

2. Cold alone does not materially affect the reduction of typhoid germs in dry earth.

3. In moist earth, although the main phenomena are the same, the destruction of the bacteria is much less rapid. With the liberal food supply introduced with the bouillon in these experiments, they appear sometimes to hold their own entirely.

TYPHOID BACILLI IN EARTH.

Series I.

Number of Tube.	February 13, 1899. Bacteria per gram.		Number of Tube.	February 14, 1899. Bacteria per gram.	
1	233000	243000	**11**	5400	1800
2	217000	—	**12**	4500	4500
3	114000	112000	**13**	7200	1800
4	123000	120000	**14**	6300	4500
5	504000	207000	**15**	2700	3600
6	107000	126000	**16**	900	5400
7	178000	141000	**17**	1800	13500
8	157000	153000	**18**	8100	9900
			19	3600	1800
			20	2700	2700
	Average	180776		*Average*	4635

Number of Tube.	February 16, 1899. Bacteria per gram.		Number of Tube.	February 20, 1899. Bacteria per gram.		Number of Tube.	February 27, 1899. Bacteria per gram.	
21	900	600	**31**	30	10	**41**	20	0
22	1000	900	**32**	10	0	**42**	30	40
23	900	200	**33**	90	50	**43**	10	0
24	600	400	**34**	0	10	**44**	0	0
25	900	1600	**35**	0	0	**45**	20	0
26	900	400	**36**	30	0	**46**	20	0
27	400	700	**37**	0	80	**47**	0	0
28	300	400	**38**	30	70	**48**	0	30
29	900	800	**39**	10	40	**49**	0	0
30	400	700				**50**	0	0
	Average	705		*Average*	25		*Average*	9

Tubes kept at 0° C.

SERIES II.

Number of Tube.	February 20, 1899. Bacteria per gram.		Number of Tube.	February 21, 1899. Bacteria per gram.	
1	4932900	5896800	**11**	130500	175500
2	5046300	4649400	**12**	103500	—
3	5216400	4876200	**13**	119700	81900
4	2286900	4706100	**14**	55800	70200
5	5953500	7030800	**15**	54000	51300
6	2570400	3628800	**16**	102600	—
7	5159700	4309200	**17**	41400	37800
8	6860700	5443200	**18**	117900	140400
9	3686500	4989600	**19**	114300	115200
			20	126900	74700
	Average	4846855		*Average*	95017

Number of Tube.	February 23, 1899. Bacteria per gram.		Number of Tube.	February 27, 1899. Bacteria per gram.		Number of Tube.	March 6, 1899. Bacteria per gram.	
21	1200	1300	**31**	30	110	**41**	340	400
22	1400	1900	**32**	210	310	**42**	160	110
23	700	1700	**33**	60	240	**43**	1770	750
24	500	1200	**34**	660	470	**44**	1070	1690
25	1100	800	**35**	110	90	**45**	160	200
26	300	700	**36**	240	290	**46**	150	900
27	2200	4000	**37**	310	500	**47**	120	800
28	2600	3100	**38**	820	210	**48**	360	430
29	1100	700	**39**	190	240			
30	400	1000	**40**	2890	2620			
	Average	1395		*Average*	525		*Average*	588

Kept at 0° C.

Series III.

Number of Tube.	February 24, 1899. Bacteria per gram.		Number of Tube.	February 25, 1899. Bacteria per gram.	
1	8541900	7144200	**11**	466200	522900
2	6747300	7200900	**12**	529200	229500
3	7314300	6066900	**13**	409500	621100
4	10432800	10092600	**14**	415800	371700
5	7711200	6917400	**15**	270900	258300
6	6860700	7597800	**16**	289800	216900
7	7881300	8278200	**17**	573300	346500
8	8731800	6577200	**18**	144900	171100
9	6463800	7711200	**19**	5400	3600
10	9695700	7994700			
	Average	7778595		*Average*	324588

Number of Tube.	February 27, 1899. Bacteria per gram.		Number of Tube.	March 3, 1899. Bacteria per gram.		Number of Tube.	March 10, 1899. Bacteria per gram.	
21	1600	1400	**31**	220	370	**41**	1710	420
22	2400	1500	**32**	380	250	**42**	1390	350
23	6900	7300	**33**	3710	370	**43**	1770	—
24	1500	900	**34**	310	—	**44**	—	—
25	1600	2600	**35**	3290	240	**45**	2870	2150
26	4000	3600	**36**	5530	2310	**46**	660	420
27	1900	1600	**37**	930	270	**47**	160	210
28	10500	25200	**38**	690	480	**48**	1510	40
			39	1020	4410	**49**	1360	1450
			40	190	140	**50**	2420	1220
	Average	4656		*Average*	1304		*Average*	1160

Kept at 0° C.

Series IV.

Number of Tube.	February 28, 1899. Bacteria per gram.		Number of Tube.	March 1, 1899. Bacteria per gram.	
1	4139100	6010200	**11**	10800	4500
2	3742200	4876200	**12**	2700	900
3	3798900	2721600	**13**	2700	1800
4	3685500	—	**14**	900	900
5	5896800	7144200	**15**	1800	2700
6	5216400	5556600	**16**	900	900
7	4025700	3628800	**17**	1800	900
			18	900	0
			19	5400	7200
			20	1800	1800
	Average	4673683		*Average*	2565

Number of Tube.	March 3, 1899. Bacteria per gram.		Number of Tube.	March 7, 1899. Bacteria per gram.		Number of Tube.	March 14, 1899. Bacteria per gram.	
21	900	1800	**31**	20	10	**41**	10	0
22	900	0	**32**	20	30	**42**	140	380
23	0	900	**33**	60	20	**43**	20	170
24	1800	0	**34**	30	10	**44**	130	110
25	900	0	**35**	90	10	**45**	350	30
26	0	0	**36**	690	60	**46**	160	60
27	0	0	**37**	340	100	**47**	40	0
28	0	900	**38**	120	20	**48**	40	20
29	0	0	**39**	250	0	**49**	190	0
30	900	0	**40**	10	10			
	Average	450		*Average*	95		*Average*	92

Kept at 20° C.

Series V.

AFTER INOCULATION.

Number of Tube.	March 15, 1899. Bacteria per gram.	
1	1045800	863100
2	1140300	989100
3	1663200	1499400
4	573300	938700
5	592200	686700
6	1297800	863100
7	1348200	—
8	1004400	919800
9	1026900	636300
10	888300	875700
	Average	939115

DAMP EARTH.

	Number of Tube.	Bacteria per gram.		Averages.
March 16	**11**	0	0	
	12	900	0	
	13	0	900	
	14	0	0	
				225
March 18	**21**	0	0	
	22	100	0	
	23	21100	25100	
	24	8400	15000	
	25	400	0	7010
March 22	**31**	220	180	
	32	10	0	
	33	3360	6580	
	34	0	10	
	35	—	—	1295
March 29	**41**	40	40	
	42	0	0	
	43	0	0	
	44	0	0	
	45	0	0	8

DRY EARTH.

	Number of Tube.	Bacteria per gram.		Averages.
March 16	**16**	900	900	
	17	9000	1800	
	18	0	2700	
	19	900	3600	
	20	0	900	2070
March 18	**26**	200	0	
	27	100	0	
	28	100	100	
	29	0	0	
	30	0	0	50
March 22	**36**	0	0	
	37	0	0	
	38	0	0	
	39	10	0	
	40	0	10	2
March 29	**46**	10	30	
	47	260	100	
	48	20	0	
	49	20	0	
	50	10	20	47

Series VI.

AFTER INOCULATION.

Number of Tube.	March 29, 1899. Bacteria per gram.	
1	1455300	1379700
2	1682100	1455300
3	1152900	1228500
4	1083600	825300
5	926100	1152900
6	1304100	1020600
7	1152900	1568700
8	1115100	1266300
9	926100	1096200
10	1398600	774900
	Average	1198260

DAMP EARTH.

	Number of Tube.	Bacteria per gram.		Averages.
March 30	**11**	1341900	1266300	
	12	2060100	1719900	
	13	1436400	1247400	
	14	2891700	3364200	
	15	963900	699300	1699110
April 1	**21**			
	22			
	23			
	24			
	25			—
April 5	**31**			
	32			
	33			
	34			
	35			—
April 12	**41**	33300	41400	
	42	900	0	
	43	1800	1800	
	44	88200	69300	
				29587

DRY EARTH.

	Number of Tube.	Bacteria per gram.		Averages.
March 30	**16**	200	300	
	17	600	4200	
	18	50	130	
	19	60	40	
	20	60	20	566
April 1	**26**	30	40	
	27	320	20	
	28	70	40	
	29	—	—	
	30	0	50	71
April 5	**36**	0	0	
	37	10	10	
	38	10	30	
	39	10	10	
	40	0	40	12
April 12	**46**	10	12	
	47	6	5	
	48	—	—	
	49	1	2	
	50	1	4	4

E. EXPERIMENTS ON THE EFFECTS OF SEDIMENTATION AND CRYSTALLIZATION DURING THE FREEZING OF TYPHOID FEVER BACILLI IN WATER.

In the experiments under Section I, the reduction effected represented simply the death-rate among the bacteria due to the adverse conditions. All the bacteria in the unfrozen water which did not perish must, from the nature of the case, be present in the thawed ice. In nature, however, the conditions are widely different. Ice is formed immediately over and in immediate contact with a large body of water. In the water, before and during the process of freezing, the bacteria, being particles somewhat heavier than water, continually tend to settle out from the region where ice is to form and fall gradually to the bottom. And when the ice formation actually takes place, a still more powerful force comes into play. In the process of crystallization there is a strong tendency to throw out all substances other than the pure compound chiefly concerned. If, then, soluble chemical compounds, other than hydrogen monoxide are excluded to a large extent when water freezes, this must be still more the case with suspended particles like the bacteria.

These *a priori* conclusions are strengthened by the work of Pengra and of the Massachusetts State Board of Health as well as by common scientific knowledge. To test them more carefully with respect to Bacillus typhi abdominalis and Bacillus coli the following experiments were made. A new wine-cask, of about ten gallons capacity, was allowed to stand full of water for a few days in order to remove any extractives present. Four pet-cocks were then screwed in, on opposite sides of the cask, two about four inches from the top and the others an inch or so from the bottom. The whole cask was jacketed with felt so that when placed at a low temperature it would freeze from above down and not from the sides inward. It was then filled with water, at about the boiling-point, drawn from an ordinary water-heater. This water was then allowed to stand for twenty-four hours, when it was found cool and still very nearly sterile, containing three or four germs per cubic centimeter. The barrel of water was then inoculated by pouring into it a bouillon culture of the germ used, the common colon bacillus in the first four experiments, the typhoid bacillus, Race B, in the last two. During the course of the experiments no sterilization was attempted beyond that partially effected by the boiling water. After adding the culture and stirring with a sterile rod, samples were taken from the four pet-cocks and planted. The covered cask was then set aside in the room or placed on a broad sill just outside the window of the laboratory, where it was exposed

to the winter's cold. After twenty-four hours of this treatment a thin sheet of ice a quarter to half an inch thick was found covering the surface. Samples were again taken from the upper cocks just under the ice, and from the lower cocks at the bottom of the barrel, and portions of the ice were also planted, being melted in sterile bottles, after washing with the water produced by their own melting, according to the usual technique.

CONCLUSIONS. 1. These experiments indicate that sedimentation does not produce marked or constant effects on colon and typhoid bacilli in water during as short a period as twenty-four hours.

2. On the other hand, the experiments show that ice formed on the surface of a quiet body of water contains only about ten per cent of the bacteria present in the water just below. This difference is probably due to the physical exclusion by the process of crystallization and not to any germicidal action, as the temperature of the ice can only differ from that of the adjacent water by a very slight amount. There are two distinct forces at work, — the low temperature, killing out germs in the ice and water nearly equally, and the crystallizing process extruding germs from the ice into the water below.

REDUCTION OF BACTERIA BY SEDIMENTATION.

B. COLI. SERIES I.

Bacteria per c.c. in samples taken from top and bottom of cask.

	December 29, 1898.				Averages.
Top	60270	51870	19320	18900	42590
Bottom	3570	3680	4550	4310	4028
	December 30, 1898.				
Top	11200	15610	12390	10095	12324
Bottom	51030	44730	13020	13580	30590
	December 31, 1898.				
Top	7070	6860	5110	5495	6132
Bottom	51870	8120	5845	40740	26640

Kept in room.

SERIES II.

	January 3, 1899.				Averages.
Top	120960	110880	114660	101430	114480
Bottom	114030	97650	103320	85050	100012
	January 4, 1899.				
Top	54180	42840	60910	56070	53500
Bottom	52920	47880	60270	62160	56050

Put outdoors. Temperature −5° to −10° C. Surface did not freeze.

REDUCTION OF BACTERIA BY SEDIMENTATION AND BY FORMATION OF ICE ON FREE SURFACE.

B. Coli. Series III.

	January 9, 1899.				Averages.
Top	28560	21630	23100	20370	23415
Bottom	25620	10010	32760	12180	20142
	January 10, 1899.				
Ice	370	250	550	670	460
Water Top	4620	4900	—	—	4380
Water Bottom	4410	10360	7490	7700	7490

Put outside. Temperature −1° C. $\frac{1}{4}$ inch ice formed.

Series IV.

	January 11, 1899.				Averages.
Top	69930	62370	45990	76860	63787
Bottom	57330	61110	68670	77490	66150
	January 12, 1899.				
Ice	1240	950	1890	780	1215
Water Top	15720	11760	9870	8410	11440
Water Bottom	8820	10920	13090	13020	11462

Put outside. Temperature, −15° C. $\frac{1}{4}$ inch ice formed.

B. Typhi. Series I.

	January 18, 1899.				Averages.
Top	147420	226800	198450	204120	194197
Bottom	247590	211680	245700	153090	214515
	January 19, 1899.				
Ice	21840	28350	27090	23940	25305
Water Top	234360	194670	147420	145530	180495
Water Bottom	209790	176660	232470	181440	200090

Put outside. $\frac{1}{8}$ inch ice formed.

Series II.

	January 19, 1899.				Averages.
Top	202230	198450	156870	171990	182385
Bottom	154980	218610	302400	254520	232627
	January 20, 1899.				
Ice	68040	75600	18480	17430	44887
Water Top	270270	404460	578340	319960	393257
Water Bottom	307180	257040	386820	238140	297295

Put outside. $\frac{1}{4}$ inch ice formed.

IV. DEDUCTIONS FROM THE EXPERIMENTS CONCERNING ICE AS A VEHICLE OF INFECTIOUS DISEASE, WITH SPECIAL REFERENCE TO THE PROBLEMS OF ICE-SUPPLY AND THE PUBLIC HEALTH.

Reviewing the several series of experiments described in detail above, and keeping carefully in mind the conditions under which natural ice is formed, cut, harvested, stored, delivered, and finally consumed, as well as those pertaining to the manufacture, distribution, and consumption of artificial ice, certain conclusions appear to be justified concerning ice as a vehicle of disease; and these conclusions are, on the whole, decidedly reassuring.

The conditions which tend naturally to purify polluted waters, are now well understood. Light, cold and poor food-supply are antiseptic or disinfectant agents of considerable power; hostile infusoria may devour the living germs of infectious disease; the chemical composition of the water may be unfavorable to their survival; and gravity may cause them to settle to the bottom, where they may soon perish for want of air. The main factor determining the reduction of germs in water is, however, the *time*, — the time during which these and other forces are left to act. Epidemiology shows clearly that disease follows best a direct, quick transfer of infectious material from patient to susceptible victim; and, if storage of water for some months could be insured, many sanitarians would consider such storage a sufficient purification.

In ice we have this condition realized, — a forced storage of at least weeks and at best of many months. At the same time the other effective conditions are also heightened. It is no wonder, then, that our experiments show a reduction of over 99 per cent in typhoid bacilli frozen; and we may be sure that in nature the destruction would exceed, rather than fall short of, such a limit.

This reduction obtains in tubes which are frozen solid, where there is no chance for mechanical exclusion. In natural ice there is another purifying influence. Of the germs remaining in the water at the time of freezing, 90 per cent are thrown out by the physical phenomena of that process. This reduction is separate from, and supplementary to, the disinfecting action of the cold. Accordingly, when both factors work together, it is obvious that only one out of a thousand typhoid germs present in a polluted stream has a chance of surviving in the ice.

Under natural conditions the pathogenic germs present in the most highly polluted stream are comparatively few. Of these few, one-tenth of one per cent may be present in ice derived therefrom. But even these scattered individuals are weakened by their sojourn under unfavorable conditions, so that, as we have seen,

they require nearly twice as long for their development as do the normal germs, and these few and weakened germs very likely could not produce many, if any, cases of typhoid fever, for vitality and virulence in disease germs are probably closely related.

With artificial ice the case is somewhat different, for such ice is made from water frozen solid, and is, as a rule, quickly consumed. Artificial ice, if made from pure water, should be above reproach; but if it be made from water that is impure it may contain the germs of infectious disease; and inasmuch as artificial ice is used quickly after its manufacture, the possibility of purification by time is excluded, and such ice might therefore conceivably be a menace to the public health.

With natural ice, as long as absolute sterilization is not effected, there must always remain a certain element of doubt, as in the use of sand filters, alluded to above, or in the practice of room-disinfection after contagious diseases. The thickness of a layer of ice is often artificially increased by cutting holes in it and flooding that already formed with the water of the pond. In such a case the effects of crystallization are excluded, as in the laboratory tubes. Ice thus formed might be cut at once, and served within a week or two; and in such an exceptional case we cannot say that sufficient of the virus might not persist to excite the malady. Yet such an instance must be very exceptional; and the general result of human experience, the absence of epidemics of typhoid fever traced conclusively to ice, the fact that cities like New York, and Lowell and Lawrence in Massachusetts, have used the ice of polluted streams, and have yet maintained low death-rates from typhoid fever, all tend to support the conclusion at which we have arrived, namely, that natural ice can very rarely be a vehicle of typhoid fever.